Maternal Productivity in Beef Cattle

The impact on the female herd of genetic selection for a divergence in fatness or feed efficiency

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BSc BVMS (Murdoch)

2010

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

School of Veterinary and Biomedical Sciences

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DECLARATION

I declare that this is my own account of my research and contains as its main content work which has not been submitted for a degree at any tertiary education institution.

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Michael Laurence

ABSTRACT

Beef cows, particularly as part of seed stock and cow/calf enterprises, are farmed extensively in temperate environments in southern Australia. The fundamental premise of a productive beef enterprise is a calving interval of 365 days. However, production system efficiency can also be determined by comparing inputs, namely megajoules of energy in the form of food, to outputs, namely the number of kilograms of beef sold each year. This thesis examined two heritable and economically desirable traits and the impact that selection to improve these traits had on beef herd productivity – defined in this thesis as *Maternal Productivity*. Selection for reduced fatness, achieved by selecting animals on the basis of low Rib Fat EBVs, can increase profitability because of consumer demand for lean meat and because sellers of cattle for slaughter are penalised if the carcasses have too much fat on them. Selection for an improvement in feed-efficiency through the trait *Net Feed Intake* (NFI) can improve profitability because it allows an increase in stocking rates or a reduction in the number of megajoules supplied to the herd for the same level of production.

There are concerns about the continued selection for these traits, particularly when energy supply is restricted such as in times of drought. There is evidence from other intensive production industries that single trait selection can compromise other desirable and necessary traits and that reduced body fatness reduces fertility in the long term. The impact of selection for fatness or feed-efficiency on *Maternal Productivity* was reported in this thesis.

Two hundred Angus heifers, selected for a divergence in either fatness (Fat vs. Lean) or feed-efficiency (high-NFI vs. low-NFI), were subjected over two breeding cycles to either a high- or a low-nutritional treatment on an extensive grazing system in the south-west of Western Australia.

Lean animals were phenotypically leaner than Fat animals at all stages of the breeding cycle and had higher predicted carcass yields than Fat animals, confirming the assumed economic benefit for selection for leanness. This result also confirmed that using Estimated Breeding Values to select for a divergence in fatness works. Low-NFI animals were phenotypically leaner than high-NFI animals at all stages of the breeding cycle and had higher predicted carcass yields than high-NFI animals. This result showed that differences in fatness between high- and low-NFI cattle previously identified in finished, grain-fed animals, persisted in the female grazing herd over two parities. There were no differences between experimental Genotypes in estimations of post-partum anoestrus interval based on fortnightly measures of blood progesterone post-calving. There were no differences between experimental Genotypes in the production measures of days-to-calving or birth, growth and weaning weights but on the low-nutrition treatment days-to-calving increased, and growth and weaning weights were lower than on the high-nutrition treatment. Although not statistically significant, there was a strong trend indicating that low-NFI animals consumed fewer megajoules of energy per kilogram of beef weaned. Nutritional treatment did not affect one particular Genotype more than the other but in all Genotypes, animals on low-nutrition consumed fewer megajoules of energy for each kilogram of calf liveweight (beef) weaned. This result suggests that selection for increased feed-efficiency will enable producers to increase stocking rates and that restricted nutrition will not decrease productivity.

Blood parameters were measured before and after calving to determine whether the Genotypes were associated with different physiological responses to nutritional restriction. No single blood parameter could be used as a marker to distinguish one Genotype from another. Beta-hydroxybutyrate and leptin were most closely associated with body condition and energy balance and differed between Genotypes when there was a difference in adiposity.

Mutations in the bovine leptin gene were examined to determine whether associations with fatness and feed-efficiency, previously reported in North American cattle, were evident in Australian cattle. The mutations were found to exist in the experimental cattle but with differing distributions and associations to those previously reported. An association between one polymorphism (E2JW) and feed-efficiency was noted but shown to be inappropriate for use as a tool in marker-assisted selection. Other associations with circulating leptin concentrations were reported.

The studies reported in this thesis showed that after two breeding cycles, *Bos taurus* cattle selected for reduced fatness or increased feed-efficiency were not compromised in terms of Maternal Productivity when nutrition is restricted. Producers can be re-assured that continued selection for these desirable traits will not impact in a negative way on the female herd. However, it must be noted that the experiment will continue for another three generations and consequently the results might change.

ACKNOWLEDGEMENTS

The good thing about starting a research PhD is that when you start you don’t know what you are in for. It is only as you spend countless hours working on the project that it dawns on you what a big undertaking it is. It is also something that cannot be done alone, and I have been very fortunate to have had a team of dedicated, committed, good natured, persistent and encouraging people to help me along the way. This is an attempt to convey to those people how grateful I am to them for everything they have done to help me with this thesis. I do, however, first need to thank and acknowledge the Beef CRC for funding the project as well for providing me with the post-graduate scholarship. Being associated with the Beef CRC post-graduate programme has been a wonderful experience and I acknowledge Graham Gardner for all his work with CRC post-graduate students.

I will start with my supervisors. Anne Barnes, my principal supervisor, is a unique and inspiring person who has made what seemed like the impossible become reality. Anne was a constant source of motivation and encouragement, who taught me to believe in what I was doing. She never gave up on me, despite my being a little behind schedule on occasion. Anne is an admirable person who knows more about most things science-related than I believed possible, and I am honoured to have been her student.

Eric Taylor and Dave Pethick have been invaluable over the last few years, providing me with guidance, advice, huge financial support, a different point of view when needed, and genuine friendship. I look forward to working with both of them in the years to come.

Without my other supervisor, Jane Speijers, I could never have finished this work. Nothing was ever too hard for Jane. Over the years Jane and I spent literally hundreds of hours on the difficult task of crunching the numbers, a task Jane performed with amazing skill, good humour and patience. I could not have hoped for a better person to guide me through the statistical quagmire that was this project.

To my colleagues at DAFWA I owe a great debt of gratitude and many have gone above and beyond the call of duty to help me. Jeisane Accioly was an unofficial supervisor but more importantly an untiring worker and dedicated project leader. She too was patient and good natured despite all those early mornings in the cold and rain, or the heat and dust. Jeisane was always supportive and I thank her for her friendship over the last four years. To my other DAFWA colleagues I say a big thank you. They include the staff at Vasse Research Centre: manager John Milligan and the beef team, Brad Seib, Tex Hahn and Ryan Drage. I also want to thank Fiona Jones, Nola Mercer, Neroli Smith and the late Bill Smart who put countless hours into the pasture work in this project, a difficult and thankless job, and I am very grateful for the excellent job they did. Thanks also to Brian McIntyre for teaching me how to scan.

In this project we had collaborators from South Australia, where the project was replicated, and from other states where members of the broader Beef CRC community were based. These include project leader John Graham from DPI Victoria, John Cooper, Mick Deland, Ian Carmichael, Nick Edwards, Michelle Herbert and Katrina Copping from Struan. All these people made this project happen and their guidance and input has been invaluable. Special mention must go to CRC Programme II leader Wayne Pitchford. Wayne went to bat for me where it counts, namely the source of the money. He made it possible for me to continue this research, fund the extensive travel and pay for the expensive lab tests. He was also a mentor, who challenged me when he thought he needed to and never stopped encouraging me, something that gave me momentum in the last stages of this work.

Others I must mention are fellow collaborators and advisors on this project and they include David Miller, Dominique Blache, Margaret Blackberry, Paul Arthur, Robert Herd, Peter Parnell, Nick Costa, Ken Chong and Chris Florides.

To my friends Dave, Jim, Chris and Steve, I say thanks for all the encouragement and guidance and for making yourselves available for a beer or to toss out an unweighted.

Finally to my family. To Mom, Dinny, George, Kerry and Sam, you know how much you mean to me and knowing that you were there for me during the tough times made it possible to keep going. I love you all very much. Special thanks to Mom for the hours spent editing this document which she may not otherwise have chosen to read so often. It has made all the difference.

To my beautiful children Jake and Abby I say that the joy of finishing this work pales into insignificance compared to the joy of having you in my life. And lastly to Nic: I couldn’t have done this without you. Thanks for making it possible for me to go on this journey, and I hope you feel it’s all worth it. Thanks for all your love and support, and for believing in me.

This thesis is dedicated to George Bester Laurence

PUBLICATIONS

1. Laurence, M., Barnes, A., Taylor, E. Pethick, D., Jones, F., Speijers, J., Accioly, J. 2009. *Maternal traits in beef cattle are not compromised by selection for leanness or feed efficiency*. Proceedings of the XI International Symposium on Ruminant Physiology, Clermont-Ferrand, 752-753.

2. Laurence, M., Barnes, A., Taylor, E. Pethick, D., Jones, F., Speijers, J., Accioly, J. 2008. *Maternal traits in beef cattle are not compromised by selection for yield or feed efficiency*. Proceedings of the Beef CRC Post-graduate Conference, Gold Coast.

3. Laurence, M., Barnes, A., Taylor, E., Costa, N., Accioly, J. 2007. *Genetic impacts on the MP of beef cattle in variable temperate environments.* Proceedings of the Beef CRC Post-graduate Conference, Bribie Island.

4. Laurence, M., Barnes, A., Taylor, E., Pethick, D., Accioly, J. 2006. *The effect of nutrition on MP in cattle selected for NFI and Fatness.* Proceedings of the Beef CRC Post-graduate Conference, Coffs Harbour.

LIST OF ABBREVIATIONS

A	author
ADG	average daily gain
AGBU	Animal Genetic and Breeding Unit
AS	accredited scanner
ATP	adenosine tri-phosphate
BCS	body condition score
BHB	beta-hydroxybutyrate
BLUP	best linear unbiased predictor
BSE	breeding soundness examination
BW	liveweight
CI	confidence interval
CL	corpus luteum
CP	crude protein
CRC	Co-operative Research Centre
DAFWA	Department of Agriculture and Food Western Australia
DEXA	dual-energy x-ray absorptiometry
DIR	direct
DM	dry matter
DMD	dry matter digestibility
DTC	days to calving
DTRS	Daughters
E	Environment
EBV	estimated breeding value
EMA	eye muscle area
FCR	feed conversion ratio
FOO	food on offer
FSH	follicle-stimulating hormone
G	gram
GF	gross efficiency
GH	growth hormone
GnRH	gonadotrophin-releasing hormone
h^2	heritability

HWE	Hardy-Weinberg Equilibrium
IGF-1	insulin-like growth factor 1
IMF	intra-muscular fat
kg	kilogram
L	litre
LH	luteinising hormone
LHRH	luteinising hormone-releasing hormone
LMM	linear mixed model
MAS	marker-assisted selection
ME	metabolisable energy
MJ	megajoule
MLA	Meat and Livestock Australia
mm	millimetres
MP	maternal productivity
NEFA	non-esterified fatty acid
NLIS	national livestock identification system
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NFI	net feed intake
NO	nitrous oxide
NPY	neuropeptide-Y
P8	P8 fatness measured at the position 8 rump site
PBBA	Performance Beef Breeders Association
PEG	Partial Efficiency of Growth
PGF2α	prostaglandin F2α
PGR	pasture growth rate
P	phenotype
P8	depth of fat measured at the position 8 rump site
PPAI	post-partum anoestrus interval
QTL	qualitative trait loci
RBY	retail beef yield
RFI	residual feed intake
RPRD	boneless retail product and lean trim adjusted to 20% fat from one carcass side
RTUS	real time ultrasound scanning
SF6	sulfa-hexaflouride
SNP	single nucleotide polymorphism
SE	standard error
US	ultrasound
VFA	volatile fatty acid
VRC	Vasse Research Centre
YG1	superior yield class

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

This thesis addresses the selection for desirable and heritable traits in beef cattle and the impact this has on breeding cows. The focus is on *Bos taurus* cattle in southern grazing regions of Australia, and pertains particularly to producers of seed stock and cow/calf enterprises. Breedplan, developed in Australia in the 1970s by the Animal Genetics and Breeding Unit (AGBU) based at the University of New England, uses best linear unbiased prediction (BLUP) technology to generate estimated breeding values (EBVs) for a range of production traits. EBVs have been used by Breedplan since 1985 (Upton, 2005) and the technology has been widely adopted by producers of beef across the country.

There are inherent concerns about the continued selection for some traits. There is evidence in other intensive production industries that single trait selection can compromise other desirable and necessary traits. Single trait selection has been shown to have detrimental results in both the dairy and pig industries (Kerr and Cameron, 1995; O'Dowd *et al.*, 1997; Kadarmideen *et al.*, 2003). In the pig industry, selection for reduction in fatness, improvement of feed efficiency and promotion of lean tissue growth to maximise finishing pig performance and carcass quality had unfavourable consequences for the long term productivity of breeding females (O'Dowd *et al.*, 1997). Similar problems were encountered in the dairy industry as a result of selection for high milk yield (Kadarmideen *et al.*, 2003; Veerkamp *et al.*, 2003).

This thesis investigates the impact of selection for leanness and feed efficiency on maternal productivity (MP). The basis of MP is a calving interval of 365 days and the term encompasses all aspects of production in the breeding herd. It includes the reproductive performance of a cow as well as factors such as breeding longevity, measured by the age of the cow when they no longer fall pregnant, and cow salvage value. When considering MP, input, particularly feed, is taken into account as well as the main output, being kilograms of live calf (beef) sold. Ultimately production per hectare is a good measure of MP, but there are other efficiency indices which are discussed in the thesis. Specifically, measures and records of conception rates, days to calving, ease of calving, birth weight, calf growth rates, milk production, weaning weight, postpartum anoestrus interval, mature cow weight (600 day weight), breeding longevity, fat storage and retrieval reflected in the change of subcutaneous fat levels at the P8 and rib sites, and cow salvage value all contribute to the understanding of MP in a breeding herd. The best measure of MP is one that includes the inputs and outputs of both dam and progeny and includes the capacity of progeny to meet market requirements (Walmsley and Parnell, 2009).

The economic desirability of the traits considered in this thesis are well recognised. Selecting for leanness in cattle is economically beneficial owing to its relationship to higher-yielding carcasses (Nkrumah *et al.*, 2004a), and net feed intake (NFI) is a trait used to measure feed efficiency in beef cattle. NFI is calculated as the actual amount of feed eaten by an individual animal less the expected amount of feed consumed based on the animal's growth rate and liveweight (Koch *et al.*, 1963). This trait is economically desirable because of the potential to reduce feed costs and increase stocking rates.

One of the main areas of concern in the beef industry regarding the selection for leanness or feed efficiency pertains to MP. The impact of long term selection for either of these two traits on the female herd is largely unknown. Some questions that have been posed by producers include:

- does selection to improve these traits change cows' fertility and production efficiency?

- are fertility and production efficiency in animals selected for a divergence in these traits influenced by level of nutrition?
- might selection to improve these traits mean fewer kg of beef are weaned per MJ of ME input?
- is there an association between circulating blood parameters or particular genetic mutations and feed efficiency or fatness?
- can cows selected for decreased fatness, or better feed efficiency, maintain good production in an energy restricted environment?

The aim of this work was to answer these questions using cattle selected for a divergence in either leanness or feed efficiency, in an extensive grazing experiment, over two breeding cycles.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Reproduction in female cattle

In a cow/calf enterprise, the ability of a breeding female to produce one calf per year is one of the key drivers of profitability. To be profitable heifers should have produced their first calf by two years of age (Stevenson, 2007). An understanding of the female bovine oestrous cycle is important when investigating any factor that may influence this annual event. A large component of MP is the fertility of the dam and its ability to conceive after calving and the following outlines the current knowledge in this area.

Female domestic cattle (*Bos taurus* and *Bos indicus*) are perennially, non-seasonally polyestrous animals, meaning that a cow will have regular oestrous cycles throughout the year. A minor exception to this is that some beef cows are still sensitive to photoperiodicity, meaning that reproductive cycling can be influenced by the number of daylight hours (Hafez and Hafez, 2000). Cattle typically reach puberty between 10 and 24 months of age (Hafez and Hafez, 2000). Puberty is variously defined as the time when a female animal first releases mature germ cells (Cunningham and Klein, 1992), the age of the first oestrous cycle (Schillo *et al.*, 1992; Bergfeld *et al.*, 1994; Boland *et al.*, 2001; Webb *et al.*, 2004), when a heifer becomes capable of reproduction (Cupps, 1991) or when a potentially fertile ovulation is followed by a luteal phase of normal duration (Stevenson, 2007). Most heifers have at least one anovulatory oestrus before the first normal cycle. The oestrous cycle is defined as all events related to reproduction that occur between two periods of sexual activity. The oestrous cycle is on average 21(14-29) days and oestrus itself is normally 18 hours but varies between 12 and 30 hours. Cattle are spontaneous ovulators, usually shedding just the one ovum per ovulation and have a gestation period of 280 (278-293) days (Hafez and Hafez, 2000). Twins, however, are reasonably common and triplets are rare.

1.1.1 The oestrous cycle

All the oocytes that will eventually ovulate exist as primordial follicles on the ovaries from birth (Hafez and Hafez, 2000). A primordial follicle is an oocyte surrounded by a single layer of squamous pre-granulosal cells (Ireland *et al.*, 2000). Once a primordial follicle is initiated it will grow until it either becomes atretic or proceeds to ovulation. The mechanisms behind the initiation and control of these primordial follicles remain largely unknown (Webb *et al.*, 2004). Follicular development is influenced by many factors but is primarily under endocrinological control.

Follicle structure

The structure of the ovarian follicle has been well described (Cupps, 1991; Stevenson, 2007) but a brief overview follows. A follicle in the initial stages consists of an outer basement membrane, the *membrane propria*. This is then lined with several layers of granulosa cells. These cells secrete the *zona pelucida,* a glycoprotein membrane that surrounds the oocyte itself. The layer of granulosa cells immediately outside the zona pelucida and surrounding the oocyte is called the *cumulus oophorus* and is surrounded by the zona pelucida. A space develops as the follicles mature between the cumulus oophorus and the rest of the granulosa cells and that contains the follicular fluids. This space containing follicular fluid is the *antrum*. Later as the follicles mature a layer of connective cells, the *thecal* cell layer, develops outside the follicle basement membrane. The thecal cells develop in two layers, the *theca interna,* and the *theca externa* which connects to the stroma of the ovary (Stevenson, 2007).

Follicle development

The development of a follicle occurs in waves (Rajakoski, 1960; Ginther *et al.*, 1989; Cupps, 1991; Ginther *et al.*, 1996; Hafez and Hafez, 2000; Webb *et al.*, 2004). Each wave is characterised by the recruitment and development of several primordial follicles, their growth and atresia, and the selection of a dominant follicle which is ultimately destined for ovulation. In cattle it is accepted that there are normally between two and three waves of follicle development prior to each ovulation (Dufour *et al.*, 1972; Ginther *et al.*, 1989; Hafez and Hafez, 2000; Ireland *et al.*, 2000; Webb *et al.*, 2004).

The initiation of the development of primordial follicles is poorly understood but believed to be growth factor dependent (Webb *et al.*, 2004). Growth factors in the Transforming Growth Factor and Insulin Like Growth Factor families are responsible for the initiation of a cohort of primordial follicles and their development into primary follicles and then into early antrum follicles (Ireland *et al.*, 2000; Webb *et al.*, 2004). Antrum follicles are larger and fewer in number and their growth is gonadotrophin dependent. Gonadotrophins are the hormones released by the anterior pituitary gland at the base of the brain and act directly on the gonads (Dyce *et al.*, 1987).

The next phase of follicle development is recruitment, where antral follicles either spontaneously regress or continue to grow and become dominant follicles. The final stages are selection and dominance, where the largest follicle undergoes the last phase of development before ovulation occurs. Figure 1-1, taken from Webb *et al.* (2004), is a schematic representation of follicle development.

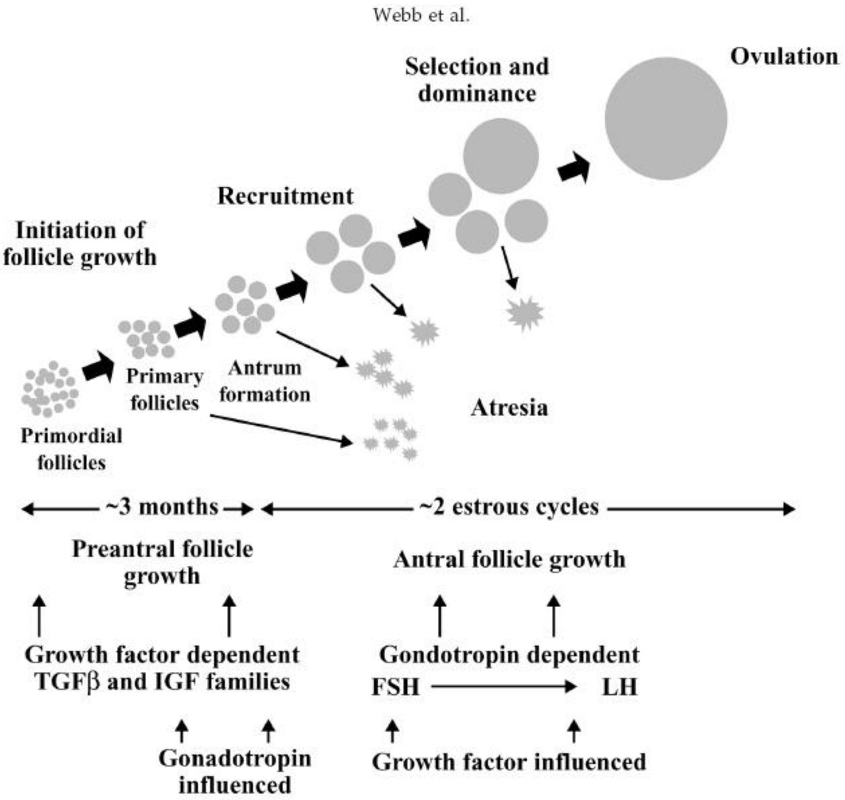


Figure 1-1: A schematic representation of follicle development (Webb *et al.*, 2004)

Gonadotrophins

The two gonadotrophins that act on the developing follicles are follicle-stimulating hormone (FSH) and luteinising hormone (LH) (Ginther *et al.*, 1989; Cunningham and Klein, 1992; Ginther *et al.*, 1996; Ireland *et al.*, 2000; Boland *et al.*, 2001; Webb *et al.*, 2004). The gonadotrophins are derived from the pituitary gland and their release is under the influence of the hypothalamus-derived gonadotrophin releasing hormone (GnRH) (Hafez and Hafez, 2000).

Follicle stimulating hormone and luteinising hormone

FSH is a glycoprotein gonadotrophin that is produced in the anterior lobe of the pituitary gland (Hafez and Hafez, 2000). It consists of a 96 amino acid alpha subunit and a 112 amino acid beta subunit and has a molecular weight of 33000 (Cupps, 1991). For a detailed outline of biochemical structure of glycoprotein hormones see Pierce and Parsons (1981). In males FSH acts on the sertoli cells in the seminiferous tubules of the testes. This action is believed to stimulate spermatogenesis but the exact mode of action is still unclear (Cupps, 1991).

In females FSH stimulates the development of primordial follicles in the ovary. It has an influence until the follicles are approximately 4mm in diameter. At this stage the follicles transfer their dependence to the other main gonadotrophin LH (Hafez and Hafez, 2000).

FSH also stimulates the granulosa cells which line the follicles to produce the steroid hormone oestrogen. This hormone has a negative feedback effect on the hypothalamus. The net effect of this is to restrict the recruitment of more primordial follicles and to promote the maturation of secondary and tertiary follicles into Graffian follicles which eventually rupture and release the ovum during ovulation.

LH is a glycoprotein hormone produced in the anterior lobe of the pituitary gland (Cunningham and Klein, 1992; Hafez and Hafez, 2000). Its release is under the influence of GnRH from the hypothalamus. LH has its main effect on the granulosa and theca interna cells of the ovary. LH is secreted from the pituitary in pulses and it is the frequency and amplitude of these pulses that governs not only the resumption of oestrus but stimulates ovulation itself (Rhodes *et al.*, 1995; Webb *et al.*, 2004). Increasing LH pulse amplitude postpartum is directly related to energy balance and is the main reason why nutritionally restricted animals have a longer postpartum anoestrus interval than well fed animals (Canfield and Butler, 1990; Wright *et al.*, 1992; Bossis *et al.*, 2000).

Inhibin

Inhibin is a protein, composed of an α and a beta subunit, which is produced by granulosa cells of antral follicles (Todoroki *et al.*, 2004). The role of inhibin is to function as a negative regulator of FSH in normal cyclic cattle (Kaneko *et al.*, 1993). As follicles mature in the ovary, circulating inhibin concentrations rise and have a negative regulating effect on GNRH leading to a decrease in FSH secretion from the pituitary gland.

Oestrogen

Each wave of follicle development is preceded by a transient increase in FSH secretion (Ginther *et al.*, 1989; Adams *et al.*, 1992; Lopez *et al.*, 2005). As the dominant follicle develops the granulosa cells that line the inside of the follicle produce oestrogen. This, as well as the production of inhibin, has a negative feedback effect on the production of FSH and limits the initiation of more primordial follicles. When the proteinaceous fraction of follicular fluid, which includes inhibin, was administered to cattle in work by Kastelic *et al.* (1990), there was a significant reduction in the concentration of circulating FSH in plasma and suppression of recruitment of cohorts of primordial follicles. As the follicles develop and become larger, through the follicular production of oestrogens and inhibins, they exert a suppressive effect on the further recruitment of follicles. Figure 1-2, taken from Ireland *et al.* (2000), shows the waves and physiological terms associated with follicle development.

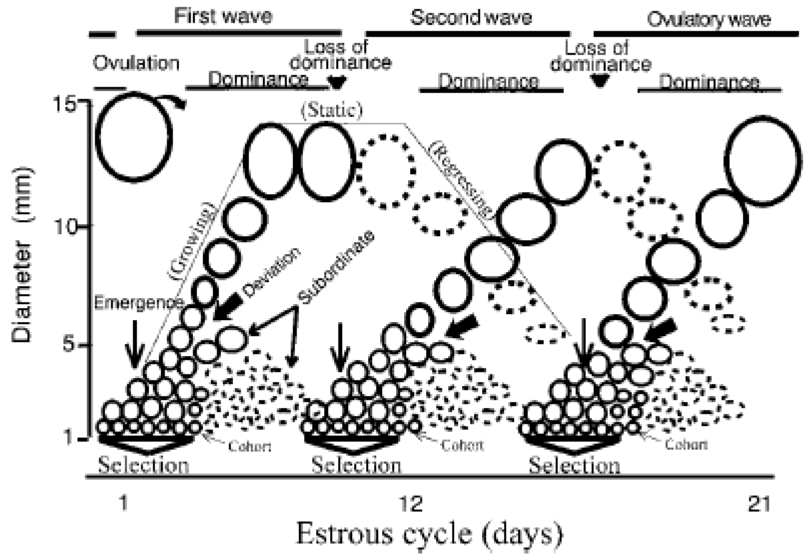


Figure 1-2: Waves of follicle development and physiological terms associated with this process (Ireland *et al.*, 2000).

Ultimately one follicle becomes dominant and transfers its dependence to LH (Ginther *et al.*, 1989; Ginther *et al.*, 1996; Ireland *et al.*, 2000; Mihm and Bleach, 2003; Lopez *et al.*, 2005). In cattle LH is secreted in episodic patterns or pulses (Rahe *et al.*, 1980) during the oestrous cycle. The pulses occur in high frequency and are of high amplitude just prior to ovulation. The amplitude and frequency of these LH spikes have a role to play in ovulation of the dominant follicle.

To summarise follicular development, follicles develop in two to three waves during a 21 day oestrous cycle in a cow. The first stage of a wave is the recruitment of a cohort of primordial follicles which after recruitment become dependent on FSH for growth. Selection is the stage where the number of recruited follicles is reduced and the follicles shift their dependency to LH. Finally dominance is when one follicle suppresses further follicular development through the production of hormones and either matures to ovulation, or becomes atretic and the waves of follicular development begin again.

Regression of the corpus luteum.

In the absence of fertilisation and attachment of the fertilised ovum, the endometrium of the uterus produces prostaglandin F2α (PGF2α). This compound is directly responsible for the luteolysis and destruction of the corpus luteum on the ovary (Cunningham and Klein, 1992). This process occurs usually about 14 days after ovulation.

1.2 Genetic improvement of cattle.

1.2.1 Animal Breeding

This thesis investigates the impact of selection for particular desirable genetic traits on the female beef herd. An understanding of some of the mechanisms behind trait selection and genetic improvement in beef cows is necessary in order to highlight the areas where knowledge of the genetic impacts is limited.

Animal breeding is concerned with the manipulation of biological differences between animals over time. Biological differences are either inherited and thus genetic in nature, or come about as a result of particular environmental influences, or more likely are a combination of the two. The phenotype of an animal is defined as the observable characteristics of an individual resulting from the interaction of its genotype with the environment (Soanes, 2008). Thus the phenotype of an animal (P) is a combination of its genetic makeup (G) and the environmental influence exerted on it (E). Hence:-

$P = f(G,E)$ (Mahner and Kary, 1997)

Selection of favourable genetic traits is one of the principal means of increasing the profitability of the beef herd (Hammond *et al.*, 1992; Kinghorn *et al.*, 1999). This increase in profitability is achieved through the manipulation of heritable biological differences between animals over time, ultimately leading to an improvement of a particular, economically beneficial, production trait or characteristic. This heritable change is cumulative over successive generations. Other means of increasing profitability include using better equipment and processes or obtaining and utilising better information about markets and types of produce (Hammond *et al.*, 1992).

History

The concept of increased profitability through genetic improvement is not a new one. Pedigrees and herd books were kept by the Englishman Robert Bakewell in the early 1800s but it was not until the 1850s that the Austrian monk, Gregor Mendel, wrote the principles of single gene genetics (Mange and Mange, 1999). Mendel's discovery of the biological laws of inheritance laid the foundations for research into genetic improvement that we still use today (Hammond *et al.*, 1992).

1.2.2 Breedplan

History

Breedplan is a system of genetic evaluation and herd recording for beef cattle breeders. The system was developed in Australia in the 1970s by AGBU based at the University of New England, Armidale, New South Wales, Australia. The unit was established in 1976 by the New South Wales State Department of Agriculture and its goal was to conduct research into the genetic improvement of livestock in Australia. Since its development, Breedplan technology has been adopted as a beef herd recording system in New Zealand, Thailand, The Philippines, The USA, Canada, Argentina, The United Kingdom and Mexico (Duff *et al.*, 2006). Similar technology is used by the pig, dairy, poultry and sheep industries.

AGBU works closely with the Co-operative Research Centre for Beef Genetic Technologies (Beef CRC) and Meat and Livestock Australia in its continuing research programmes and field training. Breedplan's genetic evaluation system uses BLUP technology to generate EBVs for a range of production traits. EBVs have been used by Breedplan since 1985 (Upton, 2005). EBVs are the technology upon which the genetic improvement of beef herds is based.

Best Linear Unbiased Predictor

First described by Henderson (1973), BLUP is a means of estimating random effects in linear mixed models and is widely used in animal breeding (Robinson, 1991). This statistical model is used because it best estimates variance components and predicts an animal's breeding value. It is used in many countries in the world to generate EBVs . The effect of an animal's breeding value as well as the effect of the environment are predicted simultaneously in this model and thus the genetic differences between herds are accounted for (Hammond *et al.*, 1992). Essentially the model estimates constants for the fixed effects (environmental effects) and predicts realised values of the random effects (breeding values). This model has become the foundation upon which herd recording and breeding value prediction systems are based around the world. Australia uses a few different systems for different species including Lambplan in sheep and Breedplan in cattle.

Estimated Breeding Values

EBVs are predictions of an animal's genetic merit, based on available performance data on the individual and its relatives (Hammond *et al.*, 1992; Duff *et al.*, 2006). The calculations are based on comparing an individual's performance to a breed base which is a historical set of data containing data on one or more traits. The breed base is collected and collated from contributing industry herds. Over time the average performance for the trait changes as a result of genetic change, and the group average for the particular year may be either above or below the breed base. The performance of an animal is compared within a contemporary group consisting of animals of the same sex and age, run in the same herd, under the same environmental conditions. Comparison can also be made between animals in different contemporary groups and of different ages through the use of pedigree linking. These links allow for adjustment for animals reared in different environments, management groups and ages.

The value assigned for a particular EBV is based on the units of measurement relevant to each trait. For example EBVs pertaining to mature cow weight are signified by the number of *kilograms* above or below the breed base that the individual is predicted to achieve. As breed bases will differ between breeds, EBVs cannot be used to compare animals of different breeds. Each year group averages are published and these tables can be used for comparing an animal's performance to the group average for the year.

Traits

EBVs are available for a range of traits. Some of these traits are not represented by EBVs in certain breeds. In other words not every breed has every EBV for every trait. Several new potential EBVs are being researched to enable producers to improve genetic performance in a broader range of desirable traits, including temperament. The following is a list of EBVs for traits used by Angus Australia, the Angus cattle breed society of Australia:

Calving ease traits – gestation length, calving ease DIR (direct), calving ease DTRS (daughters), birth weight.

Fertility traits – scrotal size, DTC.

Growth traits – 200 day weight, 400 day weight, 600 day weight.

Maternal traits – milk, mature cow weight.

Carcass traits – carcass weight, EMA, rib fat, rump fat, retail beef yield %, intra-muscular fat % (marbling).

Production trait – NFI

Carcass traits

The carcass traits are based on ultrasound scan data from live animals as well as measurements taken of animals in abattoirs at slaughter. These traits are used to select for animals to suit particular target markets. For instance selection for high intra-muscular fat will be a favourable trait if the target market is Japanese grain-fed beef. In a Breednote by Sundstrom (2002) carcass EBVs are defined. The carcass weight EBV is the estimate of genetic difference among animals in the hot carcass weight at 650 days age. Eye muscle area (EMA) is an estimate of the genetic difference among animals in eye muscle area (cm^2) at the $12^{th}/13^{th}$ rib measured by ultrasound or on a 300kg carcass. Rib fat is an estimate of genetic difference among animals in fat depth (mm) at the $12^{th}/13^{th}$ rib site measured by ultrasound or on a 300kg carcass. Rump fat EBVs are an estimate of genetic difference among animals in fat depth (mm) measured at the P8 rump site via ultrasound or on a 300kg Carcass. Retail Beef Yield % EBVs are estimates of genetic differences among animals in percentage retail beef yield in a 300kg carcass with 2-3mm fat trim and adjusted to 85% lean. Intra-muscular fat EBVs are estimates of genetic differences among animals in percentage intra-muscular fat or marbling in a 300kg Carcass. Intra-muscular fat EBVs are generated with marble score data in structured progeny test programmes.

Production traits

Researchers at the New South Wales Department of Primary industry with funding from Meat and Livestock Australia began experimental work into the area of

Feed Efficiency in 1990, and in 1994 the project was incorporated into the Beef CRC programme with the actual measuring of intakes conducted at “Tullimba” research feedlot, Trangie, NSW (Archer *et al.*, 1997).

The EBV for the trait Net Feed Efficiency or Residual Feed Efficiency is called NFI. NFI became an EBV in 2002 (Herd and Sundstrom, 2004). NFI is defined as the difference between the actual intake of an individual animal and the intake predicated on the basis of requirements for growth and maintenance. This will be explored in more detail later.

The EBV is reported as kilograms of dry matter eaten per day (kg DM/day). The figure for an individual animal will either be above or below the breed average. As such an animal with a negative number indicates that that animal is more efficient as it eats less than an average animal for the same liveweight gain (Jensen *et al.*, 1992; Arthur *et al.*, 2004; Richardson *et al.*, 2004; Crews, 2005).

1.3 Experimental traits

1.3.1 Net Feed Intake

Definition of feed efficiency

Feed efficiency is measured in different ways and is usually a comparison of intake and output traits. Historically feed efficiency has been associated with growth and body size and the challenge was to define a measure that was independent of these. The most common index of feed efficiency in beef herds is gross efficiency (GF) or its inverse, feed conversion ratio (FCR) which is widely used in feedlots (Archer *et al.*, 1999). GF is defined as the ratio between production outputs and feed inputs. Outputs are usually measured as weight gain in growing animals, so FCR is the ratio between feed intake and weight gain over a growing period (Archer *et al.*, 1999). There is a correlation between FCR and mature cow weight and thus selection for improved FCR will potentially lead to an increase in mature cow weight and as a consequence the animal will have an increased lifetime feed requirement. As such improved efficiency in the growing phase may not translate to improved overall herd efficiency and thus FCR is not the ideal measure of the efficiency of the whole beef production system.

Partial efficiency of growth (PEG) is defined as the ratio of weight gain to feed eaten after the requirements for maintenance have been extracted (Archer *et al.*, 1999). Maintenance requirements can be estimated from feeding tables such as the Standing Committee on Agriculture: Ruminant Subcommittee (SCA) (1990) or Committee on Animal Nutrition - subcommittee on beef cattle nutrition (NRC) (1996). Maintenance requirements are based on the average liveweight during the test period. PEG assumes no between-animal variation in the degree of efficiency of feed use for maintenance, which is an assumption shown to be false (Archer *et al.*, 1999).

Cow/calf efficiency is a measure of efficiency in the beef herd which is expressed in terms of *kg of calf weaned per kg of feed eaten* (Archer *et al.*, 1999). This method does not consider the feed intake of the slaughter generation or the replacement heifer generation from birth to weaning, but is still considered a good measure of herd efficiency.

NFI or Residual Feed Intake (RFI) is a measure of feed efficiency that can be applied to beef cattle. It is calculated as the actual amount of feed eaten by an individual animal less the amount of feed that animal would on the basis of its growth rate and liveweight be expected to consume (Koch *et al.*, 1963). As such NFI is a measure of feed efficiency which is phenotypically independent of growth and body size, unlike FCR or PEG (Herd and Bishop, 2000; Arthur *et al.*, 2001). More feed-efficient animals are recognised as eating a lower than average amount of feed for the same level of production and are therefore referred to as low-NFI animals. Conversely less feed-efficient animals are recognised as eating a higher than average amount of feed for the same level of production and are therefore referred to as high-NFI animals.

Heritability of NFI

Providing feed is one of the largest inputs in a beef production system so the opportunity to select animals that consume less feed is appealing to the beef-producing community. It was estimated by the United States Department of Agriculture that in 2004 and 2005 feed-associated costs made up 50.2% and 55.7%, respectively, of all non-fixed costs in US cow/calf operations (ERS, 2005). Identifying NFI as a trait has therefore provided potential for great economic benefit. NFI has been shown to be a heritable trait with estimates varying between 0.26 and 0.43 (Crews, 2005). Using the records of 1180 Angus bulls performance-tested to identify phenotypic NFI, Arthur *et al.* (2001) estimated the heritability to be 0.39 ± 0.03. More recently Nkrumah *et al.* (2007a), using 464 crossbred steers in a study into the genetic and phenotypic relationship between NFI and growth and carcass merits of beef cattle, estimated the genetic heritability of NFI at 0.42 ± 0.15 and a phenotypic heritability of 0.21 ± 0.12. They found genetic (r = 0.92) and phenotypic (r = 0.97) correlations between genetic and phenotypic NFI, indicating that both indices are very similar.

Measurement

NFI is measured in animals using an individual feed test where daily individual intakes are recorded for an animal eating a diet of known composition. The optimum duration of an NFI test is 70 days with liveweight recorded fortnightly (Archer *et al.*, 1997). More recently it was established that the duration of the test could be shortened to 63 days without reducing the accuracy of the test if liveweight was measured weekly (Wang *et al.*, 2006). These researchers from the University of Alberta Kindsella

Research Station used the GrowSafe 4000E automated feeding system which has been validated and used previously (Basarab *et al.*, 2003). These methods have proved accurate and repeatable but are time-consuming and expensive.

The search is still on for an easier way of identifying superior animals with a focus on gene and metabolic markers. One of these markers, IGF-1 was believed to be correlated to NFI (Wood *et al.*, 2004), but it has since been shown that this correlation is less than originally postulated (Johnston, 2007), so the research into a possible marker for NFI continues. NFI has recently been included in Breedplan and has its own EBV. The trait is now available to be selected for by beef producers who use Breedplan for the genetic improvement of their herds.

NFI in pasture fed animals.

There is little research available describing the measurement of NFI in mature animals on pasture (Herd *et al.*, 2003a). All phenotypic measures of NFI tend to be done on growing animals in a feedlot environment eating either a pelleted or a total mixed ration.

An experiment in the late 1990's at Trangie Research Station, NSW, compared pasture intake in high and low efficiency Angus cows in their second lactation with calves at foot (Herd *et al.*, 1998). Alkane intra-ruminal capsules were used as an indirect measure of feed intake (Dove and Mayes, 1991) and in that study they concluded that there was no difference in intake between the high- and the low-efficiency cows. However, the highly efficient animals did finish the grazing period heavier than low-efficiency animals. There was a 15% advantage in efficiency of the high efficiency cows using a ratio of calf weight at weaning to cow feed intake, but this was not statistically significant (P =0.07). The conclusion that a high-efficiency cow may be of slight economic advantage was reached owing to the greater liveweight for similar intake. There was also no difference in fatness between the two groups of animals at the end of the experiment.

Herd *et al.* (2002b) used the steer progeny of cows selected for a divergence in NFI to determine whether divergent selection of parents on post-weaning NFI was accompanied by differences in feed efficiency in steer progeny on pasture. They concluded that because the steers from the high-efficiency line grew faster, selection for NFI improves feed conversion in steers on pasture consistent with the negative regression coefficient for average daily gain with mid-parent EBVs for NFI.

Recently Meyer *et al.* (2008) did experiments to determine the effect of NFI classification on grazed forage intake of beef cows. Two experiments were conducted using first pregnant, and in the second experiment recently-calved, Hereford heifers for which NFI had been determined using the GrowSafe system. In experiment 1, 28 heifers from either a high- or a low-efficiency group were allocated to an 84-day grazing trial. Forage intake estimates were made by weekly pasture disappearance measures using a rising plate meter. Pasture quality was also determined with specific quality cuts, and animal performance records, including liveweight and condition score were recorded throughout the trial. In experiment 2, the forage intake of lactating cows was evaluated using 24 purebred heifers of known and divergent NFI, with calves at foot, on a 60 day grazing trial. The method used to measure pasture intake was similar to that used in experiment 1 and cow and calf performance data was also recorded. In both experiments the conclusion reached was that either no difference exists between low- and high-NFI beef cows grazing pasture, or methodology and small numbers limited the ability to detect any difference.

The absence of detailed research into NFI in pasture-fed, breeding cows was part of the reason for the design of the current investigation. Beef producers in Southern Australia run extensive grazing beef enterprises. The extrapolation of data and conclusions based on growing steer in a feedlot to grazing, breeding cows is potentially problematic because of the differences in physiological state, age and sex of the animals, and producers have expressed an interest in validating NFI-based research on grazing, breeding cows.

NFI and fatness

A relationship exists between NFI and fatness in young growing beef cattle but not between NFI and growth rate or mature cow weight. Herd *et al.* (2000) reported that NFI was negatively correlated to estimated lean content of the carcass (-0.22 ± 0.04) which is based on the measurement of subcutaneous back fat thickness via ultrasound at the P8 site and at the 10th and 13th ribs. Arthur *et al.* (2001) reported a genetic correlation of 0.17 between rib fat depth (mm) and NFI. Correlations of 0.3 between NFI and backfat gain, and of 0.19 between NFI and ultrasound backfat were calculated when Nkrumah *et al.* (2004a) took measurements from 150 hybrid cattle during a feedlot feed efficiency test. In a study that compared animals divergently selected for NFI, Arthur *et al.* (2005) found that there was a significant difference in fatness between the two lines but only at the time when the cows were fattest, i.e. the start of the breeding season. The link between NFI and fatness is mostly well accepted, thus selection for a reduction in NFI and therefore an increase in feed efficiency will lead to a tendency towards leanness (Davis and Simmen, 2000; Herd and Bishop, 2000; Richardson *et al.*, 2001; Johnston *et al.*, 2002; Crews, 2005). However, the linkage with body fatness and composition has recently been disputed in a study where 30 steers with divergent NFI were fed a finishing ration for 60 days (Cruz *et al.*, 2010). That study reported no differences between low- and high-NFI groups' slaughter weight, hot carcass weight, EMA, backfat or carcass fat. They concluded that NFI is not a particularly good indicator of feedlot efficiency and profitability. Another study by Herd *et al.* (1998) reported that low feed efficiency cows grazing pasture were lighter, but no fatter, than highly efficient cows. They concluded that this could imply an association of efficiency with maturity pattern.

Part of the current experiment was to determine whether the differences in fatness between high and low feed-efficient cattle detected in feedlot trials was persistent in cows grazing pasture over several generations.

Biological basis for variation in NFI

Limited research has been conducted into the biological basis for the variation in NFI but reviews by Herd *et al.* (2004) and Richardson and Herd (2004) have provided some explanation. They identified five processes that could plausibly be responsible for the variation in NFI in beef cattle. They are: variation in feed intake, digestion of feed, metabolism, activity and thermoregulation. Individually the first four processes account for only a small percentage (between 5% and 14%) of the variation but together were responsible for one third of the variation in NFI. The remaining two thirds were accounted for by differences in the processes that result in heat production and ultimately heat loss through evaporation. These processes include ion transport, protein turnover, tissue metabolism, and the biological effects of stress. The hypothesis is that stress drives some of the differences observed by Richardson and Herd (2004) among animals with either high- or low-NFI. Work by Richardson *et al.* (2002) identified that animals with high-NFI (low efficiency) were more susceptible to stress and this was reflected in particular blood cell parameters, basal levels of circulating cortisol and glucocorticoids. Increased susceptibility to stress will lead to an increase in energy wastage as a result of the associated metabolic processes, such as an increase in lipolysis and ketogenesis as well as an increase in muscle breakdown and protein turnover.

A deeper understanding of the physiological differences between high and low feed-efficient animals is required to fully elucidate the mechanisms behind the differences in feed efficiency, and possibly identify physiological markers that are indicative of more or less feed-efficient animals.

NFI and methane production

Another benefit of selecting for animals that are more feed-efficient is the potential to reduce greenhouse gas emissions from livestock. A reduction in intake is associated with a decrease in the emission of the greenhouse gas methane. Emissions of methane and nitrous oxide from manure were calculated by Herd *et al.* (2002a) using equations in the Australian National Greenhouse Gas Inventory(AGO, 1998). The animals used in the experiment were specifically bred to be divergent in NFI and the results showed that cattle selected for low-NFI produced 15% less enteric methane than those selected for high-NFI.

Using sulfa-hexaflouride ($SF_{6)}$ as a marker gas released from an intra-ruminal permeation device, Hegarty *et al.* (2005) analysed enteric methane production in steers selected for either high- or low- NFI. The results, showing enteric methane production to be significantly related to genetic variation in NFI, supported the earlier work.

In a similar study, Nkrumah *et al.* (2006) ranked 27 feedlot steers into high-, medium- or low-NFI and evaluated feedlot feed efficiency, performance, and feeding behaviour with digestion and energy partitioning. Measurement of methane emissions was done using a 4-chamber, open-circuit, indirect calorimetric system (Delfino and Mathison, 1991). NFI was correlated with daily methane production and energy lost as methane ($r = 0.44$; $P < 0.05$) and methane production was 28% and 24% less in low-NFI animals compared with high- and medium-NFI animals, respectively.

Physiological and genetic markers for NFI

Because measuring feed efficiency is expensive, time consuming and beyond the means of most producers, there has been a search to find a blood parameter or a gene marker that correlates with NFI. The blood protein IGF-1 has been identified as a possible candidate for this role (Stick *et al.*, 1998; Wood *et al.*, 2004). It has been postulated that IGF-1 may be a good physiological indicator of feed efficiency. Because of its Growth Hormone (GH)-like activity and its correlation with average daily gain, growth rate and fatness (Davis and Simmen, 1997; Stick *et al.*, 1998; Davis and Simmen, 2000) it was hypothesised that IGF-1 may be the physiological marker that functions as an indirect estimate of Net Feed Efficiency.

In the study by Stick *et al.* (1998), 36 crossbred steers were fed at three levels of intake for 84 days and their IGF-1 concentrations were measured at 28 day intervals. It was found that a positive residual correlation of 0.16 (P = .07) existed between IGF-1 and feed efficiency. The regression coefficient of the study suggested that a 1ng/ml increase in serum IGF-1 was associated with a 0.0001 kg gain/kg feed increase in feed efficiency (P = .04), supporting the hypothesised link between IGF-1 and feed efficiency.

Supporting this, Moore *et al.* (2005) measured IGF-1 concentration in 6520 Angus beef cattle in Australia at or before weaning, found that IGF-1 was moderately heritable (0.35) at weaning. This heritability has been found to be 0.11 ± 0.06 as yearlings i.e. post weaning (Graser, 2004). The genetic correlation between IGF-1 measured at weaning (average 201 days) and post weaning (average 310 days) was 1.0 ± 0.04, indicating that both measures are of the same trait (Moore *et al.*, 2005). They also found that there was a positive genetic correlation of 0.41 ± 0.21 between IGF-1 and NFI. Johnston *et al.* (2002) estimated the genetic correlation between IGF-1 and NFI after pooling data sets from the CRC and NSW Agriculture at Trangie. Across the two sites they estimated that there was a positive correlation of 0.56 ± 0.35 and 0.39 ± 0.13 between IGF-1 and NFI. As a consequence IGF-1 was commercially used to estimate NFI in British Breed beef cattle. IGF-1 is cheaper and easier to measure than NFI and measurements can be done at a younger age than a feed efficiency test can be conducted. It was suggested that selecting against IGF-1, that is for lower serum IGF-1 concentrations, would indirectly select for lower NFI and thus lead to more efficient feed conversion in the cattle.

Breedplan adopted this technology in 2004 and encouraged producers to test the IGF-1 concentrations of their young stock in order to achieve an estimate of NFI. The blood test is conducted by the Australian company Primegro Ltd, which has the exclusive right to commercialise this Australian intellectual property.

Recently the association between low IGF-1 and increased feed efficiency has been found to be weaker than originally postulated. Feed efficiency has been measured in Australia predominantly post weaning while the animal is in a growing phase as well as in older cattle closer to finishing age. These two times of measurement have led to the classification of feed efficiency as NFI-P (post weaning) and NFI-F (finishing), with the heritability estimates being 0.42 (± 0.05) and 0.9 (± 0.09) respectively. The genetic correlation between these two traits was estimated to be only 0.59 (± 0.17) suggesting that different genes influence the different traits (Johnston, 2007). Since further research has been done to establish the correlation between IGF-1 and NFI, it has been shown that IGF-1 and NFI-P have a genetic correlation of 0.17 (± 0.11) whilst IGF-1 and NFI-F have a genetic correlation of -0.22 (± 0.16), effectively making it impossible to use IGF-1 as any kind of predictor of NFI (Johnston, 2007).

Recently Lancaster *et al.* (2008) used Angus bulls and heifers divergently selected for IGF-1 concentration to evaluate the effects of post weaning serum IGF-1 selection on, amongst other things, NFI. Out of two studies they concluded that in both bulls and heifers IGF-1 selection had no effect on NFI. These results support the findings of the Australian research that has recently rejected IGF-1 as a physiological marker for NFI. Breedplan in Australia no longer recommends the measurement of post weaning serum IGF-1 to assist breeders in the evaluation of NFI in their herd.

NFI and growth

NFI was not correlated (r = 0.04) with average daily liveweight gain, which is a measure of growth rate, in work by Arthur *et al.* (2001). Richardson *et al.* (2001) evaluated the effect of selection for either high- or low-NFI on body composition. They identified the above mentioned correlation with fatness, but found no correlation between NFI and start and final feedlot liveweight, start and final eye muscle area (a measure of muscling) and daily liveweight gain.

Using data from 540 progeny of 154 British Hereford sires, collected over ten 200-day post-weaning residual feed intake performance tests conducted between 1979 and 1988, Herd and Bishop (2000) compared NFI with other production traits in British breed cattle. They found that NFI was phenotypically and genetically independent of size and growth rate. It had favourable phenotypic and genetic correlations with feed conversion ratio and was negatively correlated with estimated lean content of the carcass. Most importantly it was genetically independent of mature cow weight. They concluded that selection against NFI increases feed efficiency without changing growth rates in young animals or mature cow weights (Herd and Bishop, 2000). Later, in an Australian study of 13-month-old Angus steers entering a feedlot at, on average, 314kg, and fed for 70 days, reported that between 144 animals with low-NFI, and 165 with high-NFI, there was no difference in hot carcass weight or predicted retail beef yield (Herd *et al.*, 2003b).

Another study, using 150 hybrid cattle and comparing various measures of energetic efficiency with growth and carcass traits, showed that NFI was not related to average daily gain (r = –0.03) or metabolic weight (r = –0.02) (Nkrumah *et al.*, 2004a). These findings are important because NFI can be used to predict the feed efficiency of an animal and NFI is not affected by growth rate, mature cow weight and muscling. Previously measures of FCR and other measures of energy efficiency have been greatly influenced by growth rate and body composition gain (Nkrumah *et al.*, 2004a), meaning that selection for increased FCR would result in compromises in desirable composition traits.

NFI and maternal productivity

Little research has been done into the effects of selection for increased feed efficiency on reproductive performance in beef cattle. In one of the few studies in this area Arthur *et al.* (2005) used 185 Angus cattle, divergently selected for NFI, to attempt to evaluate the effect of the selection on MP. These researchers defined MP in three ways: (i) the changes in size and body composition of cows; (ii) reproductive performance and productivity of cows; and (iii) pre-weaning growth of the progeny of the cows. The study was conducted over three joining seasons. They found no significant difference in selection lines between pregnancy, calving and weaning rates, milk yield and weight of calf weaned. They did however identify a trend for the low-NFI animals (more feed-efficient) to calve later than the high-NFI animals. The major difference between this study and the current study was that the joinings in the three years of the Arthur *et al.* (2005) study used natural joinings in one year and then artificial breeding programmes in the other two years of their study. Using all natural joinings in a prescribed 9-week period is probably more reflective of the practices of beef producers in Southern Australia as opposed to a production system that uses artificial breeding. No measure of feed intake was attempted in the Arthur *et al.* (2005) study and therefore no conclusions pertaining to overall herd efficiency were reached. No studies have been done that provide information on the effect of nutritional stress on MP in animals selected for a divergence in NFI.

1.3.2 Fatness

Definition

Ruminant body fat can be synthesized *de novo*, mainly in adipose tissue from acetate, and to a lesser extent from lactate. It can also be formed when triglycerides are hydrolysed under the influence of lipoprotein lipase (Chilliard *et al.*, 1998a). Fat is distributed through the body in different compartments, namely abdominal (which includes peri-organ, intra-abdominal and omental fat), subcutaneous and intramuscular fat. There is also fat in the brain, bone, thorax, connective tissue and blood vessels (Dyce *et al.*, 1987). In each compartment fat is deposited in different areas and at different rates and fat mobilization occurs broadly according to the following order which is generally the inverse to the order of deposition: subcutaneous, peri-renal, omental plus mesenteric, intermuscular, intramuscular and bone (Chilliard *et al.*, 1998a). Fatness is a term used to describe the degree of adipose tissue deposition in any or all of the body compartments.

Measurement

There are several different measures of body fatness. Some are used more often in science than others. The most useful ones are those that have some relationship with the carcass traits such as RBY% and IMF %. Different measuring techniques give different evaluations of fatness. For example, total body fatness is best measured using dual-energy x-ray absorptiometry (DEXA) imaging while measuring back fat is best done using ultrasound in the live animal or carcass fat measures in the abattoir.

Measurement of fat via ultrasound at the P8 site and between the 12th and 13th rib has been shown to be closely correlated (0.74) with carcass rib fat measures (Wall *et al.*, 2004) and be an excellent predictor of Retail Beef Yield (Wolcott *et al.*, 2001). Greiner *et al.* (2003b) reported an overall correlation of 0.89 between ultrasound measures of backfat at the 12th rib site and carcass measures of backfat.

Dual-energy x-ray absorptiometry

This technology uses x-rays to determine body composition and can be used to scan whole bodies. DEXA scanners can be used to measure three body composition components – lean and fat mass as well as bone mineral. DEXA uses a radionuclide source, generated by a low current X-ray tube, which allows a high photon flux to be generated. This results in higher resolution images and, hence, precision, and a much faster scan time than the earlier Dual-Photon Absorptiometry machines. The machines generate x-rays at two energies, a detector and an interface with a computer to image the area that is being scanned. The different attenuation of the two energies by the body tissues of the subject is used to determine body composition (Laskey, 1996).

Ultrasound (US)

Ultrasound is now a readily available method for measuring fatness in live animals. Measurements are usually made with a real time ultrasound machine and a 3.5-MHz, 17cm linear array transducer placed on the skin. Real time ultrasound scanning (RTUS) has become an established technique for measuring carcass traits in live beef cattle.

Greiner *et al.* (2003b) evaluated 534 steers over a two year period to determine the accuracy of ultrasonic estimates of carcass 12th-rib fat thickness and longissimus muscle area, also known as EMA. They found that overall, correlation coefficients between ultrasound and carcass fat and longissimus muscle area were 0.89 and 0.86 respectively, and conclude that ultrasound is an accurate method of predicting carcass traits in cattle. These conclusions along with the confidence in the accuracy of the use of ultrasound to measure back fat is supported in other literature (Brethour, 1992; Hamlin *et al.*, 1995; Wall *et al.*, 2004; Schroder and Staufenbiel, 2006). Greiner *et al.* (2003b) showed that scanned rib fat depth was the most important determinant of carcass yield percentage when compared to muscle score, liveweight and scanned EMA.

It has also been shown that weight and percentage beef carcass retail product (as a representation of yield) can be accurately predicted using ultrasound and live animal measures (Greiner *et al.*, 2003a). In this research measurements of final un-shrunk liveweight (liveweight), ultrasound 12th-rib fat thickness (rib fat), ultrasound rump fat thickness (P8 fat) and ultrasound EMA are used in a formula to predict the weight of totally trimmed, boneless retail product and lean trim adjusted to 20% fat from one carcass side (kgRPRD). It was shown that 83% of the variance in kgRPRD can be accounted for using their published formula. This research concludes that live animal measurements can be reflective of potential yield in beef cattle.

Body condition score (BCS)

BCS is a subjective measure of body composition in cattle (Randel, 1990; Tennant *et al.*, 2002) and has been shown to be an accurate and repeatable method to estimate body fat and energy reserves in beef cows (Wagner *et al.*, 1988). The technique involves a visual assessment of body condition as well as a manual palpation of fat deposits on the back, pin bones, ribs, tail head, hooks and brisket (Eversole *et al.*, 2000). The system applies a numeric value to estimate the body energy reserves in the cow (Eversole *et al.*, 2000). Numerical systems often classify animals between score 1 and score 9. Tennant *et al.* (2002) used data from 3912 observations to predict the weight necessary to change BCS in Angus cattle on the 1-9 scale. They concluded that the overall weight adjustments necessary to adjust cows to BCS of 5 were (kg ± SEM): BCS = 2 (68 ± 12), BCS = 3 (50± 4), BCS = 4 (21 ± 1), BCS = 5 (0), BCS = 6(-24 ± 2), BCS = 7(-51 ± 3), and BCS = 8 (-73 ± 7). Using the 1-9 scoring system, percentage body fat has been estimated to range from 3.77% for condition score 1 to 33.91% for condition score 9 (NRC, 1996), indicting that a BCS is an accurate estimate of body energy reserves. In Australia the accepted protocol is to use a 1-5 classification system, as described in Wildman *et al.* (1982), with 1 being the leanest (emaciated) and 5 being the fattest (obese) body condition a cow may reach.

Body condition and reproduction

The relationship between body condition and reproduction is important when investigating the effect of restricted nutrition on maternal traits. Maintaining a calving to calving interval of less than one year is fundamental to the beef production system (Wiltbank *et al.*, 1962). Cows need to establish a pregnancy between 80 and 85 days after calving in order to meet the goal of weaning one calf per year (Yavas and Walton, 2000). The period when nutrition and body condition most affect the duration of the calving interval is during the postpartum anoestrus interval. Depleted energy reserves lead to a prolonged interval from parturition to first oestrus (Wiltbank *et al.*, 1962; Richards *et al.*, 1986; Wright *et al.*, 1992). Body condition at the time of calving has been shown to be the most important factor affecting the postpartum interval (Richards *et al.*, 1986; Osoro and Wright, 1992; Wright *et al.*, 1992; Rhodes *et al.*, 2003) and pregnancy rates in multiparous cows and it was suggested by Richards *et al.* (1986) that a BCS of 5 in a scale of 1-9 is the critical level affecting subsequent reproductive performance. This is supported by Meikle *et al.* (2004) who examined the effect of parity and BCS at parturition on reproductive parameters in the cow and found that lean, primiparous cows had a consistently longer postpartum anoestrus interval than fatter cows.

BCS at parturition was also shown to affect birth and weaning weight of calves (Spitzer *et al.*, 1995). This study showed that animals calving in a higher BCS had heavier calves at birth and weaning. Greater BCS at calving also led to more cows in oestrus and pregnant by 40 and 60 days of the breeding cycle. Although postpartum weight change did influence the measured parameters, it was BCS at calving that had the greatest effect. Conversely Lake *et al.* (2006) examined the effect of BCS at parturition on three year old Angus x Gelbvieh beef cow and calf performance and found no effect of BCS on first service conception rates of cows, birth weight or average daily gain of calves. But they did find that pregnancy rates were greater ($P = 0.02$) for cows in better BCS. In that study the animals were managed to achieve a BCS of either BCS of 4 ± 0.07 or BCS of 6 ± 0.07 on a 1-9 scale, a difference of approximately 100kg. These results are similar to those of an earlier study by Morrison *et al.* (1999) who managed 250 spring-calving beef cows with BCS that varied from BCS 1.5 to a BCS of 4 (BCS range 1-5), to calve in moderate (BCS 2.5) condition. They found that birth weights of calves and their weaning weights were not influenced by pre-partum BCS changes. They concluded that changes in BCS in the last trimester of pregnancy do not change the reproductive performance of cows.

Using ovariectomised cows Richards *et al.* (1991) showed that reduced energy reserves led to a reduction in circulating IGF-1 concentrations which was their postulated cause of the reduced LH secretion and thus a mechanism linking reduced body energy reserves and a prolonged postpartum anoestrus interval. This relationship is supported in other studies (Spicer *et al.*, 1990) and the increase in LH pulses have been associated with the resumption of oestrus in suckled cows (Randel, 1990; Beam and Butler, 1997; Crowe *et al.*, 1998).

Energy supply and reproduction

Suckling (Stagg *et al.*, 1998) and nutrition (Richards *et al.*, 1989; Randel, 1990; Rhodes *et al.*, 2003) are the main regulators of reproductive activity postpartum. In the study by Richards *et al.* (1989) the influence of energy supply on postpartum reproductive performance was assessed. Cows that began the experiment in good BCS developed anoestrus after 26 ± 1 weeks of feeding a restricted energy diet. Anoestrus was associated with a decrease in frequency of LH pulses. Re-alimentation of the cattle restored normal oestrous cycles and pregnancy rates. In another experiment Ciccioli *et al.* (2003) examined the influence of BCS at parturition and postpartum nutrition on reproductive performance of primiparous Angus x Hereford cows. The cows were either BCS average 4.4 or 5.1 (on a 9 point scale) and fed to gain either 0.45kg/day or 0.9kg/day for the first 71 days postpartum and then all animals were fed at the lower level until 21 days after the first oestrus. Animals fed to a higher level of nutrition postpartum had shorter postpartum anoestrus intervals and increased pregnancy rates after the first oestrus. The difference in BCS pre-calving had no effect on reproductive performance but notably the BCS difference in the study was less than 1 condition score. Lalman *et al.* (2000) fed four levels of nutrition to 36 thin (BCS 2) post-calving primiparous cows. They found that an increased plane of nutrition was associated with an increase in insulin ($P<0.01$) and IGF-1 ($P<0.001$) concentrations as well as a decrease in post partum anoestrus interval ($P = 0.04$). BCS also increased ($P<0.001$) and was correlated with IGF-1 and insulin concentrations ($r = 0.71$, $P<0.001$; $r = 0.38$, $P = 0.02$ respectively). There was also a curvilinear increase in milk yield ($P = 0.04$). Burns *et al.* (1997) found that cows fed an energy-restricted diet postpartum developed smaller ovulatory follicles and either functional or sub functional corpus lutea postpartum, and the development of a sub-functional CL was associated with lower concentrations of circulating IGF-1. Similar effects of restricted nutrition on follicle dynamics are reported in the literature (Randel, 1990; Lucy *et al.*, 1991; Murphy *et al.*, 1991; Bergfeld *et al.*, 1994; Gutierrez *et al.*, 1997; Wettemann and Bossis, 1999; Yavas and Walton, 2000).

The current experiment aims to determine whether or not restricting nutrition impacted on animals, selected for either a divergence in fatness or feed efficiency, in similar ways to that reported in previous studies.

1.4 Blood parameters

In order to characterise possible differences in the physiology of animals selected for a divergence in fatness or feed efficiency it was useful to measure circulating metabolites and hormones relating to reproduction, energy balance, nutritional status and body condition were measured. The relevance of each of the blood parameters measured in the present study is outlined below.

1.4.1 Leptin

It was proposed by Kennedy (1953) that a substance was produced by body fat to act on the brain and with a role in the control of liveweight. This “lipostatic” theory was supported by Hervey (1959) in his work on parabiotic mice, and later Coleman (1978) described obese mice, which could not synthesise the hormone leptin, and showed that they had two copies of the obese gene (ob) gene and were referred to then as *ob/ob* mice. Those mice lacked the ability to regulate weight, were hyperphagic, insulin-resistant and cold-intolerant (Coleman, 1978). He postulated that these mice lacked the ability to synthesise this lipostatic “substance”. Through positional cloning and sequencing, Zhang *et al.* (1994) first described leptin as a 146 amino acid, 16 kDa protein that had a structure similar to that of cytokines and was produced by the obesity (*ob*) gene. *Ob/ob* mice are characterized by obesity and infertility; however, when leptin was administered to *ob/ob* mice intake was decreased, weight was normalised and fertility was restored. Leptin is derived from the Greek word “leptos”, meaning “thin” and describes the hormone’s weight-reducing actions (Zieba *et al.*, 2005).

Leptin is primarily synthesised by white adipocytes (Chilliard *et al.*, 2001; Macajova *et al.*, 2004) and has a role in the regulation of appetite (Houseknecht *et al.*, 1998), reproductive performance (Hileman *et al.*, 2000) and food intake (Howie, 1999), and is associated with body composition (Fitzsimmons *et al.*, 1998; Schenkel *et al.*, 2005). Leptin circulates through the body bound to a family of binding proteins and has a half life of approximately 1.6 hours (Houseknecht and Portocarrero, 1998).

Leptin and body condition

Body fatness is the key factor that regulates adipose tissue expression of leptin as well as circulating plasma leptin concentrations (Frederich *et al.*, 1995; Blache *et al.*, 2000; Chilliard *et al.*, 2005). Body fatness, or the adiposity of an animal, reflects its nutritional history. But circulating leptin concentrations are regulated not only by degree of adiposity but also by energy intake level (Delavaud *et al.*, 2002). Delavaud *et al.* (2002) showed, in their experiments using underfed and well-fed Holstein and Charolais cattle, that plasma leptin is strongly related to adipocyte size and positively related to feeding level in adult cattle. No difference in leptinaemia existed between the breeds when leptin concentrations were corrected for adipocyte size, suggesting that leptin reflects primarily the differences in body fatness. Other research has also shown that plasma leptin increases with adiposity and is higher in females than in males (Ehrhardt *et al.*, 2000; Macajova *et al.*, 2004) and Chilliard *et al.* (1998b) showed that plasma leptin was closely linked to adipose cellularity in cattle.

Plasma concentrations of leptin were positively correlated during nutritional restriction (NR) and weight gain (WG) periods with body condition score (BCS) (r = 0.47 for NR, and r = 0.83 for WG; $P < 0.01$) and liveweight (r = 0.40 for NR, and r = 0.78 for WG; $P < 0.01$) in a study by Leon *et al.* (2004) where 24 heifers in condition score 2.6 were first subjected to nutritional restriction and then re-alimented until their BCS reached 6. Plasma concentrations of leptin decreased during nutritional restriction ($P < 0.01$) as BCS decreased. During weight gain, leptin concentration increased at BCS 3 and thereafter for each integer change in the BCS.

Leptin and level of feeding

Although body condition influences circulating concentrations of leptin, level of energy input seems to play an important role in this as well. Chilliard *et al.* (2001) suggested that changes in plasma leptin concentration were 35–50% explained by body fatness and 15–20% by feeding level. Adipose tissue mRNA and/or leptinaemia decreased significantly when energy input was restricted or when an animal was chronically undernourished. They were both subsequently increased by re-feeding (Chilliard *et al.*, 2001; Delavaud *et al.*, 2002; Chelikani *et al.*, 2004; Chilliard *et al.*, 2005). Delavaud *et al.* (2002) showed in their study that plasma leptin was positively related to plasma glucose (r = +0.52, $P < 0.01$) and negatively related to plasma non-esterified fatty acids (NEFA), (r = -0.67, $P < 0.01$). Plasma glucose is considered to be positively associated with energy intake and NEFAs are the product of fat mobilisation. Fat metabolism is the hormone induced response to negative energy balance (Tokuda *et al.*, 2002; Konigsson *et al.*, 2008).

In another study Delavaud *et al.* (2000) found that plasma leptin was decreased by 50% in underfed animals (at 40% MER) *vs.* well-fed animals (at 90% MER or ad libitum). This concurs with other research where a 50% reduction in plasma leptin was observed in growing sheep and calves which received a restricted level of nutrition for several weeks before plasma sampling (Ehrhardt *et al.*, 2000). Also, when Tokuda *et al.* (2002) fed sheep a low, moderate and high energy diet over an eight week period, they found that plasma leptin concentrations fell during the low energy phase but the increased during the high energy phase.

Leptin and feed intake

It was initially considered that leptin was only a satiety factor but it is now clear that leptin has a substantial effect on food intake. Seventy five to eighty five percent reduction in food intake was found when *ob/ob* mice were given a dose of recombinant murine leptin, and a similar effect was seen in treated lean mice (Mercer *et al.*, 1997). Administration of recombinant ovine leptin has been shown to reduce voluntary feed intake in well-fed ruminants (Morrison *et al.*, 2001). Henry *et al.* (1999) studied the effect of administering intra-cerebroventricular infusions of leptin (20 µg/h) to ewes for 3 days. They found that leptin administration reduced feed intake in a way that suggested that the action of leptin, rather than being an endocrine effect, is mediated in the brain via neuronal systems that possess leptin receptors. The brain regulates feed intake via a series of anabolic and catabolic neuropeptides (Ingvartsen and Boisclair, 2001). The catabolic neuropeptides include corticotrophin-releasing hormone, alpha-melanocyte-stimulating hormone and cocaine- and amphetamine-regulated transcript (CART) while the anabolic neuropeptides include agouti-related peptide, melanin-concentrating hormone, galanin and the orexins as well as neuropeptide Y (NPY).

The main neuroendocrine effect of leptin is that it inhibits the effects of NPY by inhibiting its synthesis in the arcuate nucleus of the brain (Stephens *et al.*, 1995). NPY is found in neurons and in their nerve terminals in many areas of the brain that are involved in intake regulation and energy balance (Ingvartsen and Boisclair, 2001). Chronic injection of NPY results in sustained increased feed intake and body fatness in rats (Zarjevski *et al.*, 1993). Hypothalamic NPY stimulates food intake and also as decreases thermogenesis and increases plasma insulin and cortisol concentrations (Zarjevski *et al.*, 1993).

It is proposed that leptin also acts as a signal of reduced feed intake because the hypothalamus senses low circulating plasma leptin concentrations and initiates a series of neuroendocrine responses (Ingvartsen and Boisclair, 2001).

Leptin and reproduction

Leptin has been shown to be an endocrine signal to the reproductive system. Leptin administration to *ob*/*ob* mice, which are infertile and lack the ability to produce leptin, increased serum LH concentrations, ovarian weight, serum levels of FSH, testicular weights and sperm counts. When leptin was injected into female *ob*/*ob* mice, these previously infertile mice ovulated and fell pregnant (Barash *et al.*, 1996).

Leptin plays a major role in the regulation of reproduction in ruminants. It is postulated that leptin plays a key role as a metabolic signal to the brain informing it of the energy status of the body (Meikle *et al.*, 2004) and thus regulating the release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Zieba *et al.*, 2005).

The main central effects of leptin on reproduction occur at the hypothalamus and the anterior pituitary gland – the hypothalamic-adenohypophyseal axis. Yu *et al.* (1997a) showed that when the anterior pituitaries of male mice were incubated with leptin for 3 hours there was a dose related increase in FSH and LH release. Similarly when median eminence–arcuate nuclear explants from the hypothalamus of the same animals were incubated with leptin, there was an increase in luteinising hormone-releasing hormone (LHRH) release at low leptin concentrations. To show that these responses also occurred *in vivo* they administered intraventricular leptin to ovariectomised mice, which led to a highly significant increase in LH, but not FSH, secretion from the pituitary gland. Their conclusions were that leptin releases luteinising hormone-releasing hormone (LHRH) from hypothalamic explants of male rats and also stimulates the release of FSH and LH from the pituitary gland *in vitro* but only LH *in vivo*. In other work they showed that LH release, induced by LHRH, is mediated by nitrous oxide (NO) and leptin acted both at hypothalamic and pituitary level to stimulate NO release by affecting NO receptors, and this induced the release of either LHRH or LH (Yu *et al.*, 1997b). Other similar work has concurred with these findings and supports the hypothesis that leptin acts centrally on the hypothalamus and pituitary to control LH secretion (Woller *et al.*, 2001).

Extrapolating from mice to ruminants, Amstalden *et al.* (2003) showed that leptin mediated LH secretion in fasted but not in well fed cows via direct action at the adenohypophysis. The conclusion that leptin has a more marked effect on LH secretion in fasted than in well fed ruminants is supported in other studies (Henry *et al.*, 1999; Maciel *et al.*, 2004). Treatment with leptin does not affect LH secretion in adequately nourished, ovariectomised ewes (Henry *et al.*, 1999) and cows (Amstalden *et al.*, 2002) but limits the reduction in frequency of LH pulses in fasted prepubertal heifers (Maciel *et al.*, 2004). The fact that leptin does not seem to stimulate an increase in LH secretion in well fed ruminants suggests that leptin stimulates the hypothalamic-adenohypophyseal axis mainly in undernourished animals (Zieba *et al.*, 2005). The reason why leptin stimulates an increase in LH pulse frequency and strength in fasted and not well fed ruminants is still unclear (Zieba *et al.*, 2005).

Leptin and parity and physiological state

Parity and time in relation to pregnancy affect circulating leptin concentrations in ruminants. This is particularly relevant for the current experiment that investigates changing concentrations of blood parameters pre- and post-calving in primiparous and multiparous cows. In dairy cows leptinaemia is high (5-9 ng/ml) in dry cows and in pregnant cows 1 month before calving (Kadokawa *et al.*, 2000; Liefers *et al.*, 2003b). It is postulated that this increase in leptinaemia is caused by the increase in adiposity in this period as well as an increase in leptin MRNA expression in adipose tissue (Ehrhardt *et al.*, 2000). However, in sheep it has been shown that the increase in leptin concentrations during pregnancy is not necessarily related to either energy balance or body fatness; rather that pregnancy is likely to be a leptin resistant state (Ehrhardt *et al.*, 2001).

There is a marked reduction in leptinaemia 4 weeks and 1 week prepartum and it reaches a nadir of 3-6 ng/ml during the first week postpartum (Blache *et al.*, 2000). Leptin then increases slightly between 3 and 5 weeks postpartum, and reaches a second nadir between 5 and 7 weeks postpartum (Kadokawa *et al.*, 2000). In late pregnancy there is a decline in dry matter intake and an increase in energy demand on the cow from the growing foetus (Liefers *et al.*, 2005) and the decline in leptin concentrations can be partly attributed to these two factors. Block *et al.* (2001) also noted a similarity between the periparturient energy balance and plasma leptin curves, linking the decreased leptinaemia to the mobilisation of body fat reserves as lactation approaches. They also suggested that there was a possible mediating effect of GH on leptin concentrations postpartum as the postpartum hypoleptinaemia coincided with an increase in circulating GH concentrations. This hypothesis was rejected by Leury *et al.* (2003) who administered GH to both late pregnant (5 and 2 weeks prepartum) and early lactating (1 and 5 weeks postpartum) cows and found that there was no decrease in plasma leptin, but that administration of insulin increased leptin concentrations. The cause of the postpartum decline in leptin concentrations is probably multi-factorial and includes the decline in adiposity and the decrease in leptin expression by fat cells and the decline in insulin concentrations. GH seems to mediate leptin concentrations only in early pregnancy (Liefers *et al.*, 2005). Short term fasting also affects leptin concentrations in different physiological states. In a series of experiments Chelikani *et al.* (2004) used post-pubertal heifers and pregnant mature cows, as well as early-lactation Holstein cattle, to show that leptin concentrations declined rapidly with short-term fasting in all three physiological states. Hypoleptinaemia was induced by fasting, most rapidly in early lactating cows and more slowly in non-lactating and prepubertal cattle. Meikle *et al.* (2004) studied the effect of parity: primiparous or multiparous, and BCS at parturition on metabolic and endocrine profiles from 1 month before to 2 months after parturition. The study used 42 (21 primiparous and 21 multiparous) Holstein cows grazing improved pasture. They discovered that primiparous, fat cows had a greater decrease in leptinaemia peri- and just post-parturition than multiparous and lean cows. They found that leptin concentrations remained low for the duration of the experimental period. Resumption of oestrus was delayed in primiparous and lean cows and they drew the conclusion that it was leptin and IGF-1 that were the endocrine signals that most probably informed the reproductive axis of body energy status.

Leptin and resumption of oestrus

In the current experiment a component of MP is the resumption of oestrous post-partum. Investigating associations between circulating blood parameters and delays in the resumption of oestrus made up part of the study. It has been shown that resumption of oestrus postpartum in cattle is possibly related to the recovery of leptin secretion postpartum. Kadokawa *et al.* (2000) used 20 high-producing Holstein dairy cows to determine the relationship between first ovulation postpartum and circulating leptin concentrations. They found that the interval to first ovulation correlated significantly ($r = 0.83$; $P < 0.0001$) with the interval from parturition to leptin nadir and any delay in recovery of leptin secretion increased the postpartum anoestrus interval. They postulated that the reasons for the change in leptin secretion in the postpartum period included 1. changes in feed intake or BCS of the cow during the postpartum period, 2. endocrine effects of parturition and lactation on adipose tissue and 3. contributions to circulating leptin by the placenta. They concluded that mechanisms behind the increase in leptin secretion postpartum are poorly understood.

Although they did not quite define a relationship between leptin concentrations and first postpartum luteal activity, Leifers *et al.* (2003b), in a similar study to the aforementioned one, did find that the recovery of leptin concentrations from the leptin nadir at parturition was related to the extent and duration of the postpartum negative energy balance, and higher leptin concentrations postpartum were associated with a shorter interval to first observed oestrus.

1.4.2 Mutations in leptin gene

Another aspect of the current investigation was to investigate the potential for mutations in the bovine leptin gene to act as genetic markers for either fatness or feed efficiency. It has been hypothesised that single nucleotide polymorphisms (SNPs) and microsatellites at different loci on the bovine leptin gene are associated with different carcass, production and meat quality traits in beef cattle (Fitzsimmons *et al.*, 1998; Buchanan *et al.*, 2002; Liefers *et al.*, 2002; Liefers *et al.*, 2003a; Crews *et al.*, 2004;

Nkrumah *et al.*, 2004b; Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005; Schenkel *et al.*, 2005; Lusk, 2007).

Five of the SNPs that have been reported in the bovine leptin gene are UASMS1, UASMS2, and UASMS3 (Crews *et al.*, 2004; Nkrumah *et al.*, 2005) in the promoter region and E2JW and E2FB in the exon 2 region (Buchanan *et al.*, 2002; Nkrumah *et al.*, 2004b) but their associations with carcass, production and meat quality traits have not been consistently verified across studies (Schenkel *et al.*, 2005).

Exon SNP

The mutation in the exon region of the bovine leptin gene E2FB, identified by Buchanan *et al.* (2002), involves a single nucleotide transition form cytosine (C) to thymine (T) and results in an amino acid change from Arginine to Cysteine. Animals are therefore either homozygous CC, TT or heterozygous CT. Schenkel *et al.* (2005) genotyped five SNPs in 1111 crossbred cattle, evaluated their interaction with the measured carcass traits fat, lean and bone yield (%) and concluded that two exon SNP (E2JW and E2FB) are associated with fat and lean yield and grade fat. They showed that for the E2FB SNP the C allele was associated with less fat yield, lower rib fat measures and higher lean meat yield than the T allele. The heterozygous genotype (CT) and the homozygous (TT) genotype had similar fat yield, rib fat measures and lean meat yield.

In Canada in 2005, when 1435 crossbred heifers and 142 steers were slaughtered the effect of the exon SNP E2FB on quality, yield and carcass weight was examined (Kononoff *et al.*, 2005). Animals with the TT genotype graded higher than both the CT and CC genotypes, and this result concurred with the finding of an association between this allele and carcass fat. However, there was a lower proportion of the TT genotype in the superior yield class (YG1) than in the CT and CC carcasses. No association with any production traits such as feed intake or feed efficiency was reported.

When the E2FB SNP was evaluated in 144 animals in a study by Nkrumah *et al.* (2004b), it was found that thymine homozygous animals (TT) had a higher rate of back fat gain, P8 fat thickness, higher carcass grade and lower yield grade and lean meat yield. The conclusion was reached that animals carrying the thymine allele versus the cytosine allele may produce carcasses with poorer grades and lower meat yields. They also showed that homozygous animals (TT) had a positive RFI (an alternative term for NFI) whereas the cytosine homozygous animals (CC) had a negative RFI. All the aforementioned results differ from those in an Australian study where 3129 animals from two separate populations were genotyped for the E2FB SNP and examined for any effect of the SNP on marbling, backfat at the P8 site and adjusted total fat (Barendse *et al.*, 2005). Although the frequency of the genotypes was the same as that reported by Buchanan *et al.* (2002) study, they found no association between the SNP and any of the fatness traits.

Promoter SNP

Three SNPs in the promoter region of the bovine leptin gene have been described (Crews *et al.*, 2004; Nkrumah *et al.*, 2005). UASMS1 at position 207 is a cytosine (C)/thymine (T) substitution, UASMS2 at position 528 is another C/T substitution and UASMS3 at position 1759 is a cytosine (C)/guanine (G) substitution.

In 2005, using a mix of fed heifers, steer and bulls (n = 1370 total) it was reported that out of the three SNPs in the promoter region of the bovine leptin gene only UASMS1 was associated with any trait, that being fat yield (Schenkel *et al.*, 2005). The C allele was associated with less fat yield with the estimate difference between the homozygous genotypes equal to -1.5% ($P<0.05$). The heterozygous genotype had similar fat yield to the CC homozygotes. There was a trend (P<0.15) for CC homozygotes to have lower rib fat measures and higher lean meat yield that the TT allele. They reported that UASMS1 and UASMS3 were completely linked and suggested that for UASMS1 all allele frequencies and associations with traits could be extended to UASMS3 (Schenkel *et al.*, 2005). This linkage was reported in another study (Nkrumah *et al.*, 2005) but in that study they reported all allele frequencies and trait associations in relation to UASMS3.

Another study specifically examined the SNPs in the promoter region of the leptin gene in 150 crossbred cattle and found that the TT genotype UASMS2 SNP was associated with a 39% increase in backfat thickness and 13% increase in marbling score (Nkrumah *et al.*, 2005). The animals with the TT genotype also showed significantly higher feed intake, growth rate and liveweight at slaughter. The T allele of UASMS2 was significantly associated with serum leptin concentrations (P < 0.001) and showed significant additive and dominance effects. TT genotype animals had higher serum leptin concentrations than CC genotype animals. Animals with the GG genotype of UASMS3 SNP showed higher feed intake and tended to have higher NFI (P = 0.06) than CG or CC animals. GG genotype animals had higher P8 measures (P = 0.04) than CG or CC animals but the different genotypes did not differ in serum leptin concentration, marbling or EMA.

Lusk (2007) examined the association of the UASMS2 SNP on backfat growth curve parameters and concluded that when 1653 animals were studied as they entered and left a feedlot, the CC genotype was heaviest at the start of the feeding period but asymptotic at the end. They also genotyped the animals for the exon 2 SNP E2FB or R25C as referred to in that study and found that SNP alone did not significantly affect growth parameters. R25C-CC/UASMS2-TT cattle exhibited the fastest backfat growth, which supports the conclusions of Nkrumah *et al.* (2005) about the association between this SNP and fatness traits as well as Nkrumah *et al.* (2004) and their conclusions pertaining to growth.

The challenge was to further investigate any association between the SNPs in the bovine leptin promoter region as well as the Exon2 SNPs, and carcass, growth and production traits in Australian cattle. No studies have investigated any association between the leptin SNP and efficiency parameters (kg weaned/ MJ ME consumed) in breeding cows.

1.4.3 Growth hormone

History

In the 1920s Evans and Simpson (1931) first characterised GH, also called Somatotropin, when they recorded the growth promoting effects of treating rats with a crude extract from the bovine pituitary. It was in 1945 that GH was isolated for the first time from the brain of mice and described as a protein hormone, containing 191 amino acids, that is synthesised in, and secreted from, the anterior pituitary gland (Li *et al.*, 1945).

The Somatomedin hypothesis

It was shown by Murphy *et al.* (1956), as cited in Etherton (2004), that quantifying incorporation of $^{35}SO_4$ into chondroitin sulfate of epiphyseal cartilage was a method of assessing cartilage metabolism and GH increased sulfate incorporation into cartilage in rats. This led Salmon and Daughaday (1957) to hypothesise that there was a serum based “sulfation” factor that mediates the growth promoting effects of GH. They showed that *in vitro* sulfate incorporation into the cartilage of rats was not stimulated by GH but that it was stimulated by the addition of serum from normal or GH treated rats. Thus it was hypothesised that a serum-based substance, probably produced in the liver, circulated in blood and mediated the effects of GH. Later in 1972, William Daughaday, in a letter to the journal Nature, proposed the term “Somatomedin” to describe this factor. The term includes both a reference to the hormonal relationship to somatotropin, or GH, as well as “medin” indicating an intermediary in somatotropin action (Daughaday *et al.*, 1972). Somatomedin was later discovered to be the same substance as the family of proteins discovered that had insulin-like actions in the body but were not suppressed by anti-insulin serum. These proteins were termed insulin-like growth Factors 1 and 2.

Since this hypothesis was first proposed it has evolved and been modified with the vast body of research that has gone into the field. It has now been shown that IGF-1 is produced not only in the liver but in most of the body’s organs and the nature of this “paracrine” action of IGF is still unclear (Le Roith *et al.*, 2001). It remains accepted that the somatic biological actions of pituitary GH are largely mediated by hepatic IGF-1.

Actions of GH

Anabolic in nature, GH has a powerful effect on nutrient partitioning between muscle and adipose tissue and changes the growth rate of these tissues dramatically (Etherton and Bauman, 1998). GH promotes lean tissue growth and reduces fat deposition in mammals via the partitioning of absorbed nutrients (Etherton and Bauman, 1998). Research using farm animals over the past three decades has shown that GH has a multitude of biological actions extending beyond increasing growth, hence the preference of some scientists for the term “Somatotropin” (Etherton, 2004). These biological actions are numerous and include increasing protein synthesis and amino acid and glucose uptake in skeletal muscle, mineral accretion during tissue growth, the synthesis of normal milk and the uptake of nutrients required for milk synthesis.

GH and adipose Tissue

In adipose tissue GH promotes a decrease in lipid synthesis in a positive energy balance state, insulin stimulation of glucose metabolism and lipid synthesis, ability of insulin to inhibit lipolysis and adipocyte hypertrophy, but it causes an increase in catecholamine-stimulated lipolysis. It also stimulates free fatty acid oxidation if an animal is in a state of negative energy balance (Etherton, 2004).

Lipogenesis and lipolysis are both affected by GH. Lipogenesis is stimulated when an animal is in positive energy balance and conversely lipolysis is promoted if an animal is in negative energy balance (Etherton and Bauman, 1998). It seems that rather than GH having any direct short term effect on adipose tissue, GH effects are chronic and change the ability of acute homeostatic signals to alter rates of lipogenesis and lipolysis.

GH appears to have a direct effect on adipose tissue, rather than being mediated via IGF-1 (Etherton *et al.*, 1993). It acts to promote lipolysis and inhibit lipogenesis in young growing animals and when the animal is in a state of negative energy balance (Etherton and Bauman, 1998; Lucy, 2008). This increase in GH secretion that is generally associated with under-nutrition is also of importance because it markedly decreases adipose tissue response to the lipogenic effect of insulin (Bauman and Vernon, 1993). GH blocks insulin-dependent uptake of glucose by adipose tissue (Lucy, 2008). There is also a high correlation in GH-treated animals between net energy balance and circulating concentrations of NEFA (Eisemann *et al.*, 1986) highlighting the lipolysis-potentiating effect of GH.

GH and carbohydrate metabolism

GH influences glucose metabolism primarily by stimulating anabolic pathways in body organs. This includes decreasing glucose uptake and oxidation by adipose tissue, decreasing the ability of insulin to inhibit gluconeogenesis in the liver, and a systemic decrease in glucose clearance and oxidation (Etherton and Bauman, 1998). During negative energy balance, such as in the postpartum dairy cow, GH concentrations can rise and this drives nutrient partitioning and supports milk production (Lucy, 2008).

1.4.4 Insulin-like growth factor-1

IGF-1 is a single-chain, 70 amino acid, basic polypeptide of 7.5 kilodaltons (Rinderknecht and Humbel, 1978). The insulin-like growth factors were first recognised in the 1970s as biologically active peptides that in general stimulated DNA synthesis, proteoglycan synthesis, glycosaminoglycan synthesis and protein synthesis (Jones and Clemmons, 1995). Their actions were identified as closely mimicking the effects of both insulin and growth hormone. The insulin like growth factors have a high affinity with insulin receptors in a host of body tissues and on most cell types. Synthesis and secretion of IGF-1 takes place predominantly in the liver and is largely under the influence of GH (Lucy, 2008). As the production of IGF-1 in the liver is directly influenced by GH (Somatotropin) the generic term “Somatomedin” has been applied to it in some literature (Thissen *et al.*, 1994). IGF-1 acts to control the secretion of GH from the pituitary via a negative feedback loop (Le Roith *et al.*, 2001). IGF-1 has GH like activity, and like GH is correlated with average daily gain, growth rate and fatness (Davis and Simmen, 1997; Stick *et al.*, 1998; Davis and Simmen, 2000).

IGF-1 and nutritional status

Circulating IGF-1 concentrations are directly related to nutritional status (Yelich *et al.*, 1995; Yelich *et al.*, 1996) and IGF-1 is a metabolic signal to the brain about the nutritional status of the animal (Richards *et al.*, 1991; Thissen *et al.*, 1994; Roberts *et al.*, 1997; Spicer *et al.*, 2002). It is recognised that nutritional restriction leads to a decrease in endocrine IGF-1 and this is the signal, along with leptin, that is most likely to inform the reproductive axis of nutritional status (Spicer *et al.*, 1990; Meikle *et al.*, 2004; Hess *et al.*, 2005).

IGF-1 and reproduction

IGF-1 plays an important role in the regulation of reproduction in cattle (Spicer *et al.*, 1990; Spicer *et al.*, 1993; Thissen *et al.*, 1994; Jones and Clemmons, 1995; Kirby *et al.*, 1996; Wettemann and Bossis, 1999; Lucy, 2000; Spicer *et al.*, 2002; Zulu *et al.*, 2002; Velazquez *et al.*, 2008). IGF-1 concentrations in blood have been associated with several cattle reproductive traits. Some of these include age at first calving (Yilmaz *et al.*, 2006), pre-attachment embryo development (Velazquez *et al.*, 2005) and age at first puberty (Velazquez *et al.*, 2008). Highlighting the importance of IGF-1 and its influence on reproduction in cattle is the fact that most of the structures of the female reproductive tract, including the ovary, oviduct, uterine horn and the developing conceptus all express the IGF-1 receptor (Velazquez *et al.*, 2008). Centrally IGF-1 receptors are found in the brain and IGF-1 enhances GnRH-stimulated LH release from cultured bovine anterior pituitary cells (Hashizume *et al.*, 2002). The size and growth rate of the dominant follicle on the ovary is associated with increased peripheral concentrations of IGF-1 (Diskin *et al.*, 2003). After parturition cows with ovulatory follicles had higher IGF-1 serum concentrations during the first two weeks postpartum than cows with anovulatory follicles (Beam and Butler, 1997). Ginreg *et al.* (1997) reported a significant relationship between days to the first ovulation post-calving and IGF-1. Greater IGF-1 concentrations in circulation reduced this interval. The extended postpartum anoestrus interval associated with low circulating IGF-1 concentrations is identified in other literature (Rutter and Randel, 1984; Rutter *et al.*, 1989; Roberts *et al.*, 1997). Bossis *et al.* (2000) reported a linear decrease in plasma concentrations of IGF-1 from commencement of dietary restriction until onset of anoestrus. This relationship between IGF-1 and nutritional status is fundamental to understanding the influence of IGF-1 on reproduction.

1.4.5 Non-esterified fatty acids and beta-hydroxybutyrate

Physiology

Plasma concentrations of NEFA are negatively correlated with energy balance in cows (Lucy *et al.*, 1991). NEFA are mobilised from adipose tissue under the influence of hormone sensitive lipase (Chilliard *et al.*, 1998a; Veerkamp *et al.*, 2003). This in turn is influenced by the hormone adrenaline. Adrenaline is released for various reasons, one of which is to initiate catabolic processes (Chilliard *et al.*, 1998a). Mobilised NEFA are metabolised in the liver. NEFA can be converted to acetyl-CoA in the liver via beta-oxidation. Some of the acetyl-CoA is fully oxidised via the tri-carboxylic acid cycle (TCA) cycle to form adenosine tri-phosphate (ATP) but excess acetyl-CoA is oxidised to form ketone bodies such as beta-hydroxybutyrate (BHB). The formation of ketone bodies in the liver occurs when excess NEFA is mobilised from body adipose reserves and the TCA cycle cannot fully metabolise the free fatty acids (Baird, 1982). NEFA can also be re-esterified to form triacylglycerols which are bound to very low density lipoproteins and transported to peripheral tissues where they are stored in lipid cohortlets (Konigsson *et al.*, 2008). NEFA concentration is an index of lipid mobilisation with a rise in NEFA indicative of a decreasing energy balance (Drackley *et al.*, 2001; Konigsson *et al.*, 2008).

NEFA, BHB and reproduction

Recently Oikonomou *et al.* (2008) evaluated the genetic relationship between BCS, NEFA, glucose and BHB, and reproduction in dairy cows. These have all been identified as indicators of energy balance. Certainly NEFA concentration is assumed to be the best indicator of a cow's energy balance because elevated NEFA concentration is the first indication of lipolysis (Reist *et al.*, 2002). Reist *et al.* (2002) concluded that BCS, BHB, NEFA recorded postpartum, and glucose concentrations measured in pregnant heifers individually had the highest genetic correlation with future reproductive performance. Measured reproductive traits included first-service conception rate, conception rate in the first 305 days of lactation, number of inseminations per conception, number of inseminations per conception for cows that conceived in the first 305 days of lactation, interval from calving to conception for cows that conceived in the first 305 days of lactation, and interval between the cow's first and second calving. Their conclusions state that selecting for blood metabolites could improve fertility and overall reproductive efficiency in dairy cows. Correlations between energy balance and various blood metabolites were also documented in that paper. They found that concentrations of NEFA correlated strongest ($r = –0.685$) with energy balance, followed by concentrations of glucose ($r = 0.457$) and BHB ($r = –0.451$).

Another study looked for associations between serum BHB and timing of first postpartum ovulation in dairy cows (Reist *et al.*, 2000). They showed that higher serum BHB concentrations during the first 6 weeks postpartum were associated with later first ovulations whereas plasma glucose and NEFA were not associated with timing of first postpartum ovulation and that maximal concentrations of BHB from parturition to first ovulation were better predictors of the onset of the oestrous cycle than mean or minima1 concentrations over the same period. Similarly Walsh *et al.* (2007) evaluated the chance of pregnancy at first service and found it to be negatively correlated with both circulating concentrations of BHB postpartum, and the duration of elevated circulating BHB.

BHB and NEFA are both useful metabolites of fat mobilisation, reflective of energy balance, and were potentially useful in the determination of differences in energy balance in energy-restricted cows, particularly after calving. Few reports have explored differences in these metabolites in beef cows selected for a divergence in NFI.

1.4.6 Glucose

Circulating glucose in ruminants is derived primarily from gluconeogenesis with only 10% of total glucose being absorbed directly from the gut (Otchere *et al.*, 1974; Young, 1977). Dietary carbohydrates are fermented in the rumen to form volatile fatty acids (VFAs) and these, particularly propionate, provide the main precursors for gluconeogenesis in the liver (Young, 1977). Glucose is the main precursor to lactose, the most abundant carbohydrate in milk, and glucose demands therefore tend to be highest during peak lactation in cows (Drackley *et al.*, 2001). Gluconeogenesis decreases during prolonged periods of under-nutrition owing to the limited supply of the main precursor propionate; however, the body attempts to compensate for this by utilising mobilised glycerol liberated from body fat stores as gluconeogenic substrates (Chilliard *et al.*, 1998a). Glucose has been shown to be positively correlated with energy balance (Reist *et al.*, 2002) and there is evidence that glucose is the main source of energy for the ovary (Rabiee *et al.*, 1999). Glucose has been shown by Vizcarra *et al.* (1998) to be influenced by BCS: a linear effect of BCS on glucose concentrations measured in post parturient cattle was reported.

Glucose is also an important intermediary metabolite in the pathways that rumen microflora use to produce volatile fatty acids (VFAs) from complex carbohydrates (Hungate, 1966; Baldwin and Allison, 1983; van Houtert, 1993).

1.4.7 Acetate

Carbohydrates are fermented by microbes in the rumen to form VFAs (Baldwin and Allison, 1983). The VFAs most commonly found in the highest concentrations in a healthy rumen are acetate, propionate and butyrate. Comprehensive reviews of the biochemical pathways and metabolic processes involved in rumen metabolism and the production of VFAs are published (Hungate, 1966; Baldwin and Allison, 1983; van Houtert, 1993). Acetate is an important metabolite in most species but has a unique place in ruminant metabolism. It is a major product of ruminal and caecal fermentation and acetate provides much of the energy requirements of ruminant tissues (Annison and Lindsay, 1961). Proportionately more acetate is derived from the fermentation of complex structural carbohydrates such as hemicellulose and pectin than from the metabolism of starch and cellulose (van Houtert, 1993) and as a consequence the ratio of acetate to propionate that is produced in the rumen is greater in animals eating roughage than in those on concentrate diets. The preferential production of acetate in roughage fed animals is postulated to be due to the substrate preferences of rumen bacteria (Hungate, 1966). After absorption through the rumen wall acetate is the only VFA that is not metabolised predominantly by the liver (Smith, 1971) but is rather used as an energy source in heart and muscle (van Houtert, 1993). The limited liver utilisation of acetate suggests that measurement of acetate in plasma is a suitable measure of acetate production. Ruminal fermentation and acetate production rise to a peak 3-6 hours after feeding (Annison and Lindsay, 1961) so measurement of plasma acetate in a well fed, grazing animal will be a good indication of acetate production in the rumen.

The other source of plasma acetate is endogenous production, predominantly via the oxidation of free fatty acids (Annison and Lindsay, 1961; Annison and White, 1962; van Houtert, 1993), a process which is generally related to the mobilisation of fat under reduced nutritional input. Other researchers have estimated the contribution of endogenous acetate to the total plasma pool. Annison and White (1962) showed that the entry of acetate after feeding was sufficiently constant to allow measurement of entry rates only during the period when a maximum concentration of rumen acetate was attained, which presumably coincided with maximum production and absorption of acetate. This translates to an animal in a well fed state. Raised concentrations of blood glucose or acetate, such as may be found in an animal on reduced feed intake, reduced the entry of endogenous acetate in sheep with emptied rumens, or in starved sheep. These results were consistent with the hypothesis that the oxidation of free fatty acids contributes substantially to the entry of endogenous acetate in sheep. Acetate measured in animals on low-nutrition is reflective of endogenous production as well as rumen-produced acetate, whereas in well fed animals the total plasma pool of acetate is derived mainly from rumen production.

In summary the blood parameters covered in this review of literature all played a role in the elucidation of the effects of restricted nutrition on productivity in cows selected for a divergence in the experimental traits. It was particularly important to measure these parameters in beef cows given the lack of beef herd relevant information. They were all either individually or together responsible for helping to gain an understanding of the impacts of both nutritional restriction and genetic selection on MP in the cows in the experiment.

1.5 General aims

In light of the current literature, the following were the general aims of the experiments reported in this thesis:

- given the reported associations of selection for reduced fatness and increased feed-efficiency with phenotypic leanness, to determine whether animals selected for high- or low-fatness, or high- or low feed efficiency, had different degrees of adiposity at different stages in the reproductive cycle, while grazing pasture and on different levels of nutrition;
- in light of the associations between leanness, or low body condition, and an increase in the PPAI, determine whether animals selected for low-fatness, or increased feed efficiency had an increased PPAI, particularly on the low-nutrition treatment;
- measure intake of green pasture in animals selected for a divergence in fatness or feed-efficiency;
- determine whether cows selected for increased feed-efficiency on the basis of a grain-based feed test, conducted at a young age, maintain the increased feed-efficiency while grazing green pasture over two breeding cycles;
- determine whether animals selected for a divergence in fatness were divergent in feed-efficiency on the basis of measures of pasture disappearance;
- determine whether animals selected for decreased fatness or feed-efficiency consumed fewer megajoules of energy for each kilogram of beef weaned per cow/calf unit;

- document, in animals selected for a divergence in fatness or feed-efficiency, the physiological responses to decreased nutrition, reflected in measures of blood hormones and metabolites;
- determine whether any particular blood parameter was appropriate for the use as a physiological marker for feed-efficiency or fatness.
- examine the distribution of SNPs in the bovine leptin gene, previously reported in North American studies in the Australian experimental cattle;
- determine if particular SNP/s were associated with carcass traits or feed-efficiency and
- whether these SNPs could be used as a tool for MAS in beef cattle.

CHAPTER 2. GENERAL MATERIAL AND METHODS

2.1 Animals

All experimental work was approved by the Murdoch University Animal Ethics Committee as well as the Research Quality Management System of the Department of Agriculture, Fisheries and Food, Western Australia. All animals remained on site at Vasse Research Center (VRC), Busselton, Western Australia for the duration of the experiments.

All animals were stud Angus cattle from Breedplan registered herds. The animals were from two sources in Australia and were imported to VRC in two cohorts; one in 2006, comprising 2005-born animals (1^{st} cohort) and one in 2007, comprising 2006-born animals (2^{nd} cohort). The animals were sourced from either Trangie Research Station, NSW, 147°58'45"E, 32°2'0"S, or from stud Angus, Breedplan registered, industry herds in the South West of Western Australia.

Trangie animals

These cattle, henceforth referred to as NFI animals, were bred at Trangie Research Station, Tullimba, NSW. This is a NSW Department of Primary Industry research centre. The herd of origin at Trangie had been selected for a divergence in NFI for 5 generations. The animals that made up the 1^{st} and 2^{nd} cohorts of animals sent to VRC are a product of the fifth generation of divergent selection for NFI.

2006

In 2006, 120 animals were selected from the Trangie herd and transported to Western Australia. Selection was on the basis of the mid-parent EBV for NFI for each animal. Sixty animals had mid-parent EBVs for high feed efficiency (the low-NFI animals), and 60 animals had mid-parent EBVs for low feed efficiency (the high-NFI animals). Section 9.3 lists the Trangie animals in the experiment and their genetic information is contained within this list.

The animals were transported by truck and arrived at VRC on 25 March 2006**.** All quarantine procedures and requirements as set out by the Department of Agriculture, Fisheries and Food were met (DAFWA, 2008). Upon arrival the animals were kept in a quarantine paddock for 30 days and fed hay *ad libitum.* The individual feed test designed to determine the actual NFI of the animal (Archer *et al.*, 1997) was begun in May 2006. After the animals had undergone an individual feed test to determine their actual NFI (details described below), and after the joining period, thirty pregnant animals with the highest actual NFI and thirty pregnant animals with the lowest NFI were selected for the experiment. The final pregnancy diagnosis and allocation was done on 23 May 2007.

2007

In 2007 a further 70 animals, 35 high-NFI and 35 low-NFI based on mid-parent EBVs, were selected and transported to VRC where they arrived on 10 February 2007. These animals underwent an individual feed test and were joined in September 2007. Fifteen pregnant animals with the highest actual NFI and fifteen pregnant animals with the lowest NFI were selected for our experiment. The final pregnancy diagnosis and allocation to replicates was done on 5 May 2008.

2.1.1 Western Australian Industry Animals

These animals, henceforth referred to as Industry animals, were selected on the basis of their mid-parent EBVs for Rib Fat. The herds of origin were all Breedplan-registered, stud Angus herds in the South West of Western Australia.

2006

Thirty six animals with EBVs for extremes of fatness (the Fat animals), and 56 animals with EBVs for extremes of leanness (the Lean animals), were selected and imported to VRC in April 2006. Animal information is contained within Section 9.4. The animals were transported to VRC on 12 April 2006 and subjected to all quarantine requirements and protocols (DAFWA, 2008). After the animals had been joined with the bulls their pregnancy status was established via ultrasound and manual pregnancy diagnosis techniques (details follow), and 30 pregnant fat animals and 30 pregnant lean animals were allocated to replicates and became part of the experiment. The final pregnancy diagnosis and allocation was done on 13 June 2007.

2007

In 2007 a further 28 Fat animals and 30 Lean animals were purchased from similar herds of origin as the 1st cohort. The animals were transported to VRC on 3 March 2007 and subjected to all quarantine requirements and protocols (DAFWA, 2008). These animals were joined in September and pregnancy tested in December 2007. After a final pregnancy diagnosis the heifers were allocated to replicates in March 2008. In this year 24 were also sourced from Trangie Research Centre and subjected to the same quarantine procedures described above.

For a timeline of important events in the experiment see Appendix 9.1.

2.2 Individual feed test

The NFI animals were tested to determine their actual feed efficiency. All testing took place at VRC. The 1st cohort was tested between 23rd May and 31st August 2006 and the 2nd cohort was tested between 8th May and 17th August 2007. There were 120 animals in the 1st cohort tested in 2006. The animals were selected and allocated to

96 individual testing pens based on their mid-parent EBV for NFI . Thirty animals with the lowest, and 30 animals with the highest parental average EBV for NFI were allocated to individual pens. Thirty six animals were then allocated at random to the remaining pens. The 24 remaining animals were allocated to the group pens in three groups of eight. In 2007 the 2nd cohort of NFI animals (n=60) underwent the test procedures in the individual pens.

Figure 2-1: Shed used for individual NFI feed test. Vasse Research Centre, Busselton WA.

Figure 2-2: Individual pens used for NFI feed test.

The individual feed test ran for 90 days which included an adaptation period, and was based on the NFI test described in Archer *et al.* (1997). In 2006 the diet consisted of a total mixed ration of 30% oaten hay, 30% triticale, 20% rolled oats, 15% lupins, 2% mineral mix, 1% urea, 1% ground limestone, 0.5% salt and 0.5% gypsum. The diet was prepared at VRC. The diet was 89.3% dry matter, contained 14.3% crude protein, had an Metabolisable Energy (ME) of 10.5 MJ/kg and a dry matter digestibility (DMD) of 73.3. In 2007 an export pellet, “825 Export Pellets”, prepared by Wesfeeds Pty Ltd of 31 Sevenoaks Street in Bentley WA, was used as the total ration. The ration contained 20.9% crude protein, had an ME of 10.6 MJ/kg, a dry matter of 90% and had a DMD of 72.6%.

An adjustment period of 14 days occurred at the start of the test where the animals were gradually introduced to the ration as ever-increasing proportions of a ration/hay mixture. By day seven the animals were on 100% ration and were on this for another seven days before the actual test period began. This then ran for a further 76 days.

In the individual pens, the feed bins for each animal were weighed before and after the addition of the ration each day in order to obtain daily intake measures. Residue was cleaned out once a week. For the group pens the feed was added to a feed bin periodically and bins were checked regularly and were never empty. Automatic recording took place when an animal fed from the bin. The software would register the NLIS electronic ear tag of the animal and record a before and after weight for the feed bin. The machine used for the electronic measurement was Ruddweigh Feed Intake Recorder (Feedlot Monitor, Ruddweigh, Falconer Street, Guyra NSW 2365). The feed information downloaded to a central computer daily and from this the daily intake measure for each animal could be calculated. One pen had ongoing technology failures and the eight animals in this pen were excluded from the experiment because not enough data to calculate actual NFI had been collected.

The animals were weighed every seven days on scales within the testing pen area. This procedure took no more than one hour and was done between 7 and 10 am. Each animal was away from its pen for no more than 30 minutes. Feeding took place after 10 am every day.

2.3 Allocation of animals to the experiment

2.3.1 2007

Table 2-1 shows the allocation of the animals to the experiment. The methodology for the allocation of the animals is described below.

NFI animals

NFI animals were chosen for the experiment on the basis of their EBV for NFI as it was recorded in Breedplan on the 12 December 2006. This EBV included in its calculation the result of the NFI feed test conducted at VRC. The 30 most and the 30 least efficient animals were chosen from the 1st cohort of NFI heifers and used in the experiment. Only pregnant animals were chosen for the experiment. The high-NFI animals were assigned to two nutritional treatment groups, high- and low-nutrition. Each treatment had two replicate groups, one of eight and one of seven animals. The animals were allocated to replicate groups so that each replicate group had a similar average EBV for NFI.

Each replicate was physically located on a different farm. The replicate group of eight were housed on Farm A while the replicate of seven was housed on Farm B. The same method of allocation was applied to the low-NFI animals.

Industry animals

In 2007 Industry animals were chosen for the experiment on the basis of their EBV for rib fat as it was available from Breedplan in March 2007. This EBV at this time included one actual measure of the animal's rib fat depth, taken in January 2007. The 30 animals with EBVs for the greatest extremes of fatness, and the 30 animals with EBVs for the greatest extremes of leanness were chosen from the 2006 cohort of animals and used for this experiment. Only pregnant animals were chosen for the experiment.

The Fat animals were allocated to two nutritional treatment groups, high- and low-nutrition. Each treatment had two replicate groups, one of eight and one of seven animals. The animals were allocated to replicate groups so that each replicate group had a similar average EBV for rib fat. Each replicate group was physically located on a different farm. The replicate group of eight animals was housed at Farm A while the replicate group of seven was housed on Farm B. The same method of allocation was applied to the Lean animals.

For the allocation of all animals in 2007, every attempt was made to ensure that the average EBV for mature cow weight, across all replicate groups, was as similar as possible. The average liveweight and stage of pregnancy at the time of allocation was as similar as it was possible to achieve.

2.3.2 2008

NFI animals

In 2008 the 2nd cohort of animals was added to the experiment. The 20 most and the 20 least efficient animals, on the basis of their EBV for NFI available in January 2008, were chosen from the 2nd cohort of NFI heifers and added to the experiment. Again only pregnant animals were chosen for the experiment. In this year the high-NFI animals were again allocated to either a high- or a low-nutrition treatment. They were then allocated to two replicate groups of five animals each. The first replicate group of 2nd cohort animals joined the replicate group of cattle from the 1st cohort on Farm A for the duration of the grazing season. This meant that the replicate groups from the 1st and 2nd cohorts were running together in the same mob. This was done to increase stocking rates of the mobs to enable more control of the restricted grazing aspect of the experiment. The other replicate group of the 2nd cohort joined the replicate group of the 1st cohort on Farm B and ran as one mob for the same reasons. The same method of allocation was applied to the 2nd cohort of low-NFI animals.

Industry animals

In 2008 a 2nd cohort of Industry animals was chosen and added to the experiment on the basis of their EBVs for rib fat available from Breedplan in January 2008. This EBV calculation included one measure of the animals' rib fat depth, taken in January 2008.

The 20 animals with EBVs for the greatest extremes of fatness, and the 20 animals with EBVs for the greatest extremes of leanness were chosen from the 2nd cohort of animals and added to the experiment. Again only pregnant animals were allocated to replicate groups. In this year the Fat animals were allocated to either a high- or a low-nutrition treatment. They were then allocated to two replicate groups of 5 animals each. The first replicate group of 2nd cohort animals joined the replicate group of cattle from the 1st cohort on Farm A for the duration of the grazing season. This meant that the replicate groups from the 1st and 2nd cohort were running together in the same mob. This was done to increase stocking rates of the mobs to enable more control of the restricted grazing aspect of the experiment. The other replicate group of the 2nd cohort joined the replicate group of the 1st cohort on Farm B and ran as one mob for the same reasons. The same method of allocation was applied to the 2nd cohort of Lean animals.

Table 2-1: The allocation of 200 animals to the experiment

Nutritional treatment		high				low			
Replicate group/location		1/Farm A		2/Farm B		1/Farm A		2/Farm B	
cohort		1st	2nd	1st	2nd	1st	2nd	1st	2nd
NFI	high	8	5	7	5	8	5	7	5
	low	8	5	7	5	8	5	7	5
Industry	Fat	8	5	7	5	8	5	7	5
	Lean	8	5	7	5	8	5	7	5

Table 2-2 is a summary of the EBVs for the experimental traits used at the time of allocation of the animals to the experiment.

Table 2-2: Experimental trait EBVs – midparent (mid-p) and at time of allocation (allocation). Rib fat EBV units mm fat, NFI EBV units kg/head/day. Standard errors in parenthesis.

	1st cohort				**2nd cohort**			
	high-nutrition		low-nutrition		high-nutrition		low-nutrition	
Traits	mid-p	allocation	mid-p	allocation	mid-p	allocation	mid-p	allocation
Fat (rib fat EBV)	1.24 (±0.12)	1.14 (±0.2)	1.19 (±0.09)	1.46 (±0.25)	1.06 (±0.13)	1.04 (±0.12)	1.32 (±0.09)	1.10 (±0.15)
Lean (rib fat EBV)	-1.33 (±0.08)	-1.49 (±0.10)	-1.26 (±0.08)	-1.62 (±0.10)	-1.01 (±0.10)	-0.81 (±0.13)	-1.09 (±0.04)	-0.84 (±0.12)
high-NFI (NFI EBV)	0.72 (±0.04)	0.69 (±0.05)	0.72 (±0.05)	0.66 (±0.05)	0.51 (±0.14)	0.76 (±0.10)	0.40 (±0.12)	0.70 (±0.10)
low-NFI (NFI EBV)	-0.51 (±0.04)	-0.59 (±0.05)	-0.44 (±0.05)	-0.54 (±0.04)	-0.22 (±0.18)	-0.84 (±0.11)	-0.36 (±0.18)	-0.82 (±0.14)

2.4 Joining

2.4.1 2006

In 2006, twelve West Australian stud Angus industry bulls were used to mate the 1st cohort of animals. Their ages ranged from 433 to 886 days on 1 September 2006, which was the joining start date. All bulls had average EBVs for growth. Bulls were subjected to a Breeding Soundness Evaluation (BSE) which included semen testing and a modified serving capacity test. The BSE was used to identify first- and second-line bulls. First-line bulls were those with no abnormalities in the categories of conformation, semen quality and libido, while second-line bulls had less than perfect results in one of these categories but were still able to be used in a breeding programme. Bulls with serious abnormalities in any of these categories were culled. It has been shown that BSE can identify bulls that will ultimately impregnate more females than bulls with sub-satisfactory BSE results (Farin *et al.*, 1989). The BSE was not an estimate of future reproductive potential as the bulls were still young and had not reached their full reproductive capacity. The aim was to identify the yearling bulls which were most willing and able to serve the maximum number of heifers at the time of joining.

The semen test was conducted using electro-ejaculation to collect a semen sample. Each bull was restrained in a crush for this procedure. This was then examined under a microscope immediately. A subjective assessment of semen quality was made by a skilled operator and included an assessment of concentration, and motility. A sample was then fixed in buffered formal saline for morphological examination in the laboratory. Two hundred sperm were assessed under a phase contrast microscope and graded according to appearance (Barth and Oko, 1991).

Serving ability test

These tests are based on the practice of using restrained heifers to asses the joining pro ratio and libido of a bull, as originally designed by Blockey (1981a) and later modified and described by Blockey (1981b). The serving capacity test was used to determine if a bull could satisfactorily mount and serve a heifer. It was also used to identify any penile abnormalities such as lateral deviation or corkscrew penis. Eight non-pregnant, cycling heifers that were not part of the current experiment were injected with two doses of PGF2α (Lutalyse, Pfizer Animal Health, APVMA number 38700) ten days apart, and two days after the second dose the heifers were used in the serving capacity test to coincide with the onset of oestrus. Three heifers were roped into and restrained in testing crates (Custom made, Murdoch University, Perth, Australia) in a rectangular yard thirty metres long by fifteen metres wide. Bulls were released into the yard two at a time and successful joinings were counted. A subjective assessment of libido was made at the same time and the bulls were ranked in order according to libido and the ability to serve. No time limit was set on the test because the aim was simply to identify if the bulls were able and willing to serve. Therefore, one successful joining led to the removal of the particular bull from the yard and the introduction of a new bull. The heifers were released after approximately three services and replaced by unjoined and in oestrus heifers.

Joining procedures

The first cohort of animals was joined in groups of 35. The heifers were divided into six groups of 35 with 2 bulls per group. This provided for an initial bull: female of 1:17 – approximately 6%. The optimal bull: heifer yearling bulls is 1:25 (Healy *et al.*, 1993). There have been studies into the benefits of single versus multiple sire joinings with conflicting results (Farin *et al.*, 1982; Lunstra and Laster, 1982), but because of the limited paddock space available it was decided to compromise and used groups with a maximum of 2-3 bulls per group. Bulls were rotated though the groups every three weeks. Ultimately each heifer was exposed to 6 bulls during the joining period which began on 1 September 2006 and progressed for 9 weeks.

2.4.2 2007

In 2007 a further 20 bulls were included in the joining programme. These bulls were sourced from Trangie in NSW and were selected for a divergence in NFI. Upon arrival at VRC all bulls were subjected to a BSE and identified as first- or second-line bulls. Where possible only first-line bulls were used in the joining programme with second line bulls acting as spares in case of accident or injury.

Joining procedures

In 2007 year the 1st cohort was joined in replicate groups with one bull per replicate group. Bulls were rotated every two weeks during the joining period which ran from 3 September 2007 for 9 weeks. As the bulls were now specifically either Industry (Western Australian) bulls or high-NFI or low-NFI (Trangie) bulls, an effort was made to expose all cows to at least one bull of each classification. It was also ensured that as some bulls and cows were sourced from the same producer, no siblings were joined to each other during the joining period. Each replicate was exposed to three bulls during the joining period. The 2nd cohort was joined in groups of 35 with two bulls per group. The bulls were rotated every 3 weeks. Ultimately each group was exposed to six bulls during the joining period.

2.4.3 2008

The 2008 joining programme was very similar to the previous years' programme. No bulls were added to the programme and all animals were joined in replicates with one bull per replicate. Bulls were rotated every two weeks during the joining period which ran from 5 September 2008 for 9 weeks.

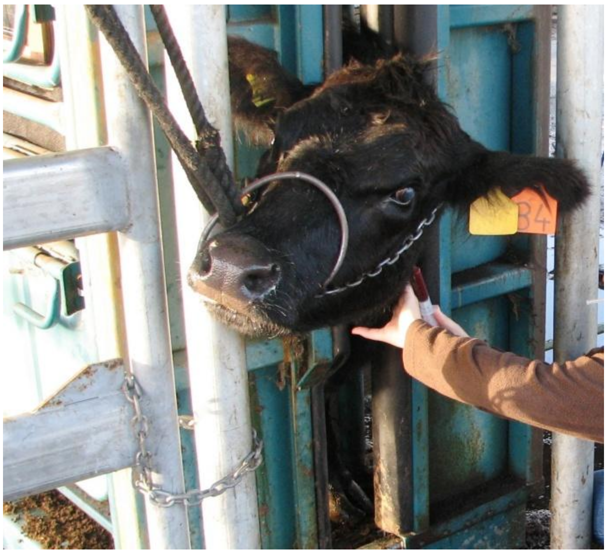
2.5 Liveweight measurement

Live liveweight (BW) was measured using a Ruddweigh Weigh Scale Systems (Galagher Australia PTY Ltd, Sydney, Australia) electronic weighing system and load cells until February 2008. At that time the machine was changed to a TruTest XR 3000 with Software: Link 3000 software interface (TruTest Ltd, Manukau, New Zealand). Changing weight-recording technology was a management decision taken by staff at VRC but the change did not alter the accuracy of weight recording because weights of cattle, measured using both machines, were compared on a day and found to be the same. Animals were let through a race and individually weighed on the load cells. Weighing took place between the hours of 0700 and 1100.

2.6 Collection of blood samples

Blood samples were collected using a 20ml syringe and 1.5 inch 18 gauge needle (BD PrecisionGlide™ Needle, Becton Dickinson Pty Ltd, Singapore) via venepuncture of the jugular vein. Animals were restrained in a cattle crush and the head retracted to one side using a custom made steel ring halter (see Figure 2-3**)**. Average collection time from restraint to needle withdrawal was fifteen seconds. Blood was collected into a syringe and emptied into 9ml plastic tubes containing lithium heparin (BD Vacutainer®, Becton Dickinson Pty Ltd, New South Wales, Australia), or 9ml EDTA (BD Vacutainer®, Becton Dickinson Pty Ltd, New South Wales, Australia). Blood samples were placed on ice immediately after blood collection. Lithium heparin tubes were subsequently centrifuged at 3000 rpm for 15 minutes. The plasma supernatant was pipetted into 1.5ml plastic, push cap tubes (Interpath Services PTY Ltd, Heidelberg West, Victoria, Australia, Catalogue number - S4411UU) and frozen for future batch analysis. This plasma was used for all hormone and biochemical analyses. Blood collected into EDTA tubes was frozen and used for DNA extraction.

Figure 2-3: Ring halter used for the collection of jugular blood samples



2.7 Pregnancy diagnosis

Pregnancy diagnosis was done by the author and other registered and experienced veterinary surgeons using a combination of manual palpation and ultrasound assisted diagnosis. Manual palpation was conducted at a minimum of thirty five days after the end of the joining period. Trans-rectal ultrasound using an Aloka SSD 500 machine with 5 MHz linear probe was conducted at a minimum of 21 days post removal of the bull. The following calving season showed the combination of these two methods to be 100% accurate.

2.8 Live animal ultrasound techniques

US was used to measure subcutaneous fat deposits at the Position 8 (P8) site and between the 12th and 13th rib. It was also used to measure EMA and IMF. This procedure was done at regular intervals during the year. The procedure was done with the live animal standing in a crush with the operator standing on the left hand side (see Figure 2-4). The ultrasound machine was a Pie Medical Scanner 200 RTUS machine equipped with a 17.2-cm, 3.5-MHz linear transducer probe and was used to obtain the measurements. The author was responsible for the majority of the scans for the subcutaneous fat measures but an accredited scanner was responsible for a bi-annual full herd scan. This scan included measurements of EMA and IMF.

To validate the results of the authors' technique a full herd scan was performed by the author 20 days prior to a full herd scan performed by the accredited scanner. A shorter time interval between scans was not logistically possible. One hundred and ninety five animals were scanned on each occasion and there was a highly positive correlation ($R^2 = 0.84 \pm 0.24$) between the authors results and the results from the accredited scanner. The X1 variable coefficient was 0.84 ± 0.03; this being not significantly different from zero indicates that on average the two operators achieved the same result for an individual animal.

Figure 2-4: The author taking US subcutaneous fat measurements from a live animal using a linear transducer probe with an ultrasound machine

2.9 Calving.

In 2007 the first calf was born on 6 June and the last calf was born on 10 August. In 2008 the first calf was born on 26 May and the last on 15 July. During the calving period each replicate group was checked by a VRC staff member every morning for new born calves or for animals that were close to parturition.

2.9.1 Birth weight recording

The birth weight of each calf was recorded a maximum of 16 hours after birth. A leather sling and a clock face scale (QWM/Accuweigh Pty Ltd**,** Geebung, Queensland, Australia), mounted on a frame on the tray of a farm utility vehicle was used to restrain and weigh the calves (see Figure 2-5). The calves were weighed in the paddock in which they were born. Each calf had its sex and weight recorded, and was ear-tagged with a sequentially numbered ear tag. Male calves were castrated using Elastrator

Rings© (Nasco International, Fort Atkinson, Wisconsin, USA) placed at the base of the scrotum.

Figure 2-5: Measuring calf birth weight using vehicle-mounted sling and scales

2.9.2 *Obstetrical problems*

Animals that were deemed to be having difficulty calving were examined by a veterinary surgeon. Animals were examined in a crush. Obstetrical problems were corrected by delivering the calf either with traction, or in a severe case, via caesarean section. All animals were given epidural anaesthesia prior to these procedures. A 1.5 inch, 18 G needle (BD PrecisionGlide™ Needle, Becton Dickinson Pty Ltd, Singapore) was inserted in the epidural space caudal to the sacrum and 5ml of Lignocaine 20 (Ilium Veterinary Products), a 20mg/ml Lignocaine hydrochloride solution, introduced into the space.

Caesarean section was performed under local anaesthetic, in the standing position, where a flank incision was made with a scalpel in the left flank of the animals and the calf removed from the uterus via this incision. Closure of the uterus was done using a continuous, inverting, suture pattern of absorbable suture material (Braun Aesculap metric 6). Muscle layers were closed with a simple interrupted layer of absorbable sutures. Skin was closed using nylon (Braun Aesculap metric 6) in a simple interrupted pattern. Skin sutures were removed after three weeks. Animals that were subjected to this procedure received analgesic injections as well as long acting antibiotics, administered by injection.

2.9.3 Post-calving collection of blood samples

In both the 2007 and 2008 calving season, two weeks after the first calf was born in a replicate, the group was walked to the closest handling facility between 0700 and 1000 on a day of the week designated to be the sampling day for that replicate. Any cow with a calf a minimum of two weeks old had a blood sample collected as described above. All cows and calves were weighed at this time. The same replicate was gathered again on the same day of the week, a fortnight after the initial sampling. A sample was collected from every cow that had calved, and each cow was weighed between two and three weeks post-calving and thereafter approximately every fortnight. Blood samples were not collected from cows that had not calved. Cows had blood samples collected between one and eight times during the post-calving sampling period, depending on their calving date. The post-calving blood sample collection period ended one week before the 2007 joining period and in 2008 ended at the end of the joining period.

All experimental animals had US measures of fat taken during a two-week period in the middle of the calving period in 2007, and monthly in the 2008 post-calving period.

2.10 Statistical analyses

The experiment was a 4 x 2 randomised block design with the 4 experimental Genotypes consisting of high-NFI, low-NFI, Fat and Lean, and 2 nutritional treatments; high-nutrition and low-nutrition. Linear mixed models (LMM) which included treatment effects, covariates and appropriate random effects were used to analyse all the data. Covariance structures were defined for random terms as required and simplified where likelihood ratio tests indicated that this was possible Hierarchical tests (Type I sums of squares) and a 5 % level of significance were used to assess whether treatment and covariate effects were significant. When covariates were fitted after treatment effects they explained within-treatment variance; when they were fitted before treatment effects, treatment effects were adjusted for covariates.

Predicted treatment means and standard errors (SE) were corrected to mean covariate values where appropriate. In general, Genotype means were not corrected for those covariates that were thought to be part of the Genotype effect, and nutrition means were adjusted for all covariates. Where there were no interactions between Genotype and nutrition, main effect means have been presented for each Genotype and for each nutritional treatment.

Occasionally, results were transformed to the log scale for the purposes of the analysis so that the assumption of constant variance made within the LMM framework was valid. Predicted means and Standard Errors (SE) were produced on the log scale but means were re-transformed to present in graphs in the results section of the chapter. In these cases a 67% confidence interval (CI) on the re-transformed scale is presented to indicate variability of the mean as this is the same CI represented by the usual error bar, ±SE.

Specific statistical models varied depending on the y-variate and are described in detail in the relevant chapters. All statistical analysis was done using the REML procedures in GenStat 11th edition (VSN International Ltd, Hertfordshire, UK).

2.10.1 Genotype definition

In this experiment the animals were defined by one of four Genotypes. Industry animals were classified as *Fat* or *Lean* based on their selection for a divergence in EBV for fatness. In order to select animals that represented the top or bottom 10%, animals with Mid-Parent EBV for rib fat at the extremes were selected (see Appendix 9.4). Similarly the NFI animals were classified as *high-NFI* or *low-NFI* based on their selection for a divergence in NFI EBV. This was done by selecting animals from the Trangie research herd with mid-parent EBV for NFI at the extremes (Appendix 9.5), in order to select animals that represented the top or bottom 10% of animals. Statistical models used throughout this thesis are used to look for main effects of *Genotype* and *nutrition* on various variates. Comparisons between the four Genotype categories – Fat, Lean, high-NFI and low-NFI – were subdivided into a comparison between Industry and NFI animals (line), a comparison between Fat and Lean animals, and a comparison between high- and low-NFI animals. The effect of line is not of interest in this experiment and is not referred to when results are presented. The linear effect of EBV within line was not examined because the experimental design required selection of extremes of EBV's which resulted in a concentration of EBVs in the top or bottom 10%. The experiment was designed to test differences between extreme EBV groups which were grazed in separate paddocks.

2.10.2 Interpretation of bar charts

In this thesis Genotype and nutrition main effect means are often presented using bar charts. When Genotype means are presented there is a separation between *lines of animals*, i.e. Industry animals (Fat/Lean) are clearly separated from NFI animals (high-NFI/low-NFI) by a bold line on the bar chart. This is done because the intention is not to compare Genotype effects between, but rather within lines, and the same bar chart is used for convenience.

The other important point to consider when viewing bar charts is that means from both years of the experiment (2007 and 2008) are presented on the same graph. However, the statistical models have been applied only to results from one particular year and therefore when interpreting significant differences between means, only significant differences between means *within a year* are annotated. An assessment of significant difference between means in different years can be attained by comparison of error bars but the difference has not been statistically tested and thus is not annotated as such on the chart. Truncated y-axes are used to give a better impression of variation. Error bars on bar charts represent either standard errors or 68% Confidence Intervals. Each chart specifies which method is used. These are included to give an impression of variation around each mean. They are not 5% Least Significant Differences and thus comparison of error bars does not always indicate significant ($P < 0.05$) differences between means.

2.10.3 Fixed and random terms

Fixed and random terms used in the LMMs are described below:

Fixed terms

line – is part of the main effect of Genotype and represents the comparison between Industry animals and NFI animals.

FatvsLean - is part of the main effect of Genotype and represents the comparison between the Fat and Lean Industry animals.

HiNFIvsLoNFI - is part of the main effect of Genotype and represents the comparison between the high-NFI and low-NFI animals.

nutrition - represents the comparison between the high-nutrition treatment and the low-nutrition treatment.

line.nutrition - represents the interaction between line and nutritional treatments. Any term in the model which takes the form of *term.term* is describing the interaction between the two terms. There are occasionally three-way interactions in some of the models and take the form of *term.term.term*.

location - represents a comparison between Farm A and Farm B.

cohort - represents the comparison between 1^{st} and 2^{nd} cohort of animals (used only in the 2008 analysis).

height - the height (measured to the wither) of the dam measured less than 4 months before calving. *Height* was used as a covariate to adjust for any difference in frame size of the dams between lines.

calving date - the date of birth of the calf.

calf sex - the sex of the calf.

dam birth date - the age of the dam.

3^{rd} trimester P8 - P8 fat depth during the 3^{rd} trimester of pregnancy.

lactating - whether a dam is lactating or not in the post-calving period. Dams that had still-born calves or lost calves postpartum are described as *not lactating* in the analysis. Neonatal deaths all occurred within three weeks of birth.

days-post-calving - the number of days after calving that a sample was taken. These values were different for every dam because of the different calving dates.

pre-calving measure - the value for the sample taken in the last trimester of pregnancy.

multiple birth - represents the comparison between single and multiple births (only used in the 2008 analysis as there were no twins born in 2007).

birth type - male calf, female calf or twins (used only in DTC analysis in 2008 because no twins were born in 2007).

%lact - the percentage of cows that were lactating in a replicate group (used in the analysis of efficiency parameters). *%lact* was used as a covariate in the analysis of efficiency parameters to correct for different numbers of lactating animals in a treatment. This would inherently influence the group intake measures for each treatment.

av calving date - the average calving date of a replicate group. *Average calving date* was used as a covariate in the analysis of the efficiency parameters to correct for the effect of the spread of calving dates on intake measures across a replicate group.

Random terms

replicate group - represents the groups of animals that remains constant throughout the experiment and moves physical location as a group.

dam ident - represents the individual identification numbers of the dams.

The significance of all fixed terms in LMMs used in this thesis are shown in Appendix 9.6.

2.11 Animal health

Management of the breeding herd required various routine animal health related procedures to be performed. These included:

2.11.1 Routine vaccination of all heifers and cows

Heifers all received a course of two injections of Ultravac 7 in 1 Vaccine (Commonwealth Serum Laboratories Limited, Australia) to induce immunity against *Leptospira borgpetersenii* serovar *hardjo*, *Leptospira interrogans* serovar *pomona*, *Clostridium perfringens, Cl. tetani*, *Cl. septicum, Cl. novyi* type B and *Cl. chauvoei*. Two 2.5ml doses were given subcutaneously in the ischiorectal fossa, 4 weeks apart, in April of the year the animals arrived at VRC. In subsequent years the animals received a single 2.5ml dose which acted as a booster.

Heifers were also given a course of two injections of Pestiguard Vaccine (Pfizer Animal Health) to induce immunity against Bega and Trangie isolates of Australian Bovine Viral Diarrhoea Virus. Two 2ml doses were given subcutaneously into the ischiorectal fossa, four weeks apart, a minimum of six weeks before joining start date each year. In subsequent years each animal was given a 2ml dose, six weeks before joining start date, as a booster.

2.11.2 Routine vaccination of bulls

In the year of their arrival at VRC, bulls were given a course of two injections of *Ultravac 7 in 1 Vaccine* (Commonwealth Serum Laboratories Limited, Australia) to induce immunity against *Leptospira borgpetersenii* serovar *hardjo, Leptospira interrogans* serovar *pomona, Clostridium perfringens, Cl. tetani, Cl. septicum, Cl. novyi* type B and *Cl. chauvoei*. Two 2.5ml doses were given subcutaneously in the ischiorectal fossa (Colazo *et al.*, 2002), 4 weeks apart, in April of the year the animals arrived at

VRC. In subsequent years the animals received a single 2.5ml dose which acted as a booster.

In the year of their arrival at VRC, each bull was given a course of two injections of Vibrovax Vaccine (Pfizer Animal Health) to induce immunity against *Campylobacter fetus* subspecies *venerealis*, biotypes *venerealis* and *intermedius*. Two doses of 5ml were given into the ischiorectal fossa, four weeks apart and at least four weeks before the joining start date. In subsequent years each bull was given one 5ml dose at least four weeks before joining start date as a booster.

2.11.3 Anti-parasitic treatment

All cattle were treated once a year, in May, for external and internal parasites with 0.5 mg/kg Cydectin (Moxidectin, Triclobendazole) pour-on (Fort Dodge, New South Wales, Australia).

2.11.4 Trace Elements

In April of 2008 it was identified by assay of serum glutathione peroxidise that animals may have been deficient in Selenium. Every animal was therefore treated with Selpor Selenium Pour On (Virbac Australia Pty Ltd) at a dose rate of 2ml per 50kg liveweight of a 5g/L topical selenium solution.

2.12 Nutritional treatments

Two nutritional treatments were imposed in this experiment, high-nutrition and low-nutrition. Animals on high-nutrition were provided with approximately 1.2 times the maintenance requirements of energy and protein for an animal of the average liveweight and physiological state of the treatment. Industry animals were larger and heavier than NFI animals, and therefore the treatment averages were split into those two lines. Animals on low-nutrition were provided with 80% of the maintenance requirements of an animal of the average liveweight and physiological state of the Genotype. The treatments included three discreet periods over the course of each year of the experiment: 1. Grazing green pasture; 2. Grazing dry pasture; 3. Supplementary feeding.

A note on the nutritional treatments

The aim of the experiment was to subject the experimental Genotypes to a divergence of energy supply with the low-nutrition treatment providing on average 80% of the cows' maintenance requirements for their physiological state. However, welfare guidelines set out for this experiment decreed a cut-off point for nutritional intervention. This was set at BCS 1.5 with a requirement to intervene if any individual animal fell below that point. The nature of the design therefore decreed that when one animal fell below that point, within a line, all animals in not only the replicate group but in the whole nutritional treatment had to be supplemented until that critical animal reached BCS1.5 again. Because of biological variation in BCS and differences in physiological state (pregnant, lactating etc.) there was usually a large variation of BCS within a replicate group and thus intervention often resulted in some if not most of the animals in a replicate group being supplemented when their BCS and energy balance was not as low as the experimental design called for. This was a problem throughout the experiment and was one of the postulated reason for the absence of nutritional impacts when it was hypothesised that there may be some.

2.12.1 Grazing green pasture

2007

The 1st cohort was allocated to replicate groups on the 23rd May and assigned a specific paddock for the duration of the green pasture grazing season. An accepted rule of thumb for the South West region of Western Australia is that the stocking density for breeding cows is approximately 1 cow/calf unit per hectare, (personal communication (McKlay and McGreggor, 2006). The experiment paddocks were either 3.6 hectares for the low-nutrition treatment or 6.3 hectares for the high-nutrition treatment. Table 2-3 shows the stocking rate for each year of the experiment.

Each paddock was divided into eight sub paddocks using electrified tape, with each sub paddock having access to a water source. The animals spent 4 days in one section and were then moved to the adjacent section for another 3 days, resulting in a 28 day rotation. It became clear early in July 2007 that using this rotational grazing system would not restrict nutrition adequately to reach the nutritional divergence required of the low-nutrition treatment. This was due to the limited grazing pressure on each section of the paddock and an un-seasonal winter flush. The rotational grazing system was abandoned for a set stocked grazing system whereby animals on the low-nutrition treatments grazed only in paddocks with 500kg DM/hectare on offer and animals on high-nutrition grazed in paddocks with over 1500kg DM/ hectare on offer. Reducing the food-on-offer (FOO) in some low-nutrition paddocks was achieved by introducing a steer herd for a short period. This method restricted the available feed for the low-nutrition animals and a better divergence was achieved between the high- and low-nutrition treatments. BCS and BW were monitored fortnightly with the target differential between the high- and low-nutrition regimens being 20%. It must be noted and considered throughout this thesis that the post-partum management of the nutritional treatments in 2007 was interpolated with a period of unregulated pasture availability to all animals. This occurred during an un-seasonal winter flush when it was made clear that the original design was failing and the combination of unseasonably warm weather, exponential pasture growth and low stocking pressure meant that animals on the low-nutrition treatment were, for approximately six weeks in July, exposed to higher energy supply than the experimental design dictated. This had its consequences as will be noted in the experimental chapters. One purpose of the low-nutrition treatment should have been to put the cows into a state of negative energy balance post-calving, but in reality the energy deficit at this time was less extreme because of the unexpected conditions.

Table 2-3: Stocking rate (cow/calf units per hectare) in each experimental year (2007 and 2008). Numbers of units per paddock is the average of the two replicate groups (7.5 in 2007, 12.5 in 2008).

year of experiment	**cow/calf units/hectare**	
	high-nutrition 6.3 hectares	low-nutrition 3.6 hectares
2007 (7.5 cow/calf units per paddock)	1.19	2.08
2008 (12.5 cow/calf units per paddock)	1.98	3.47

2008

Because of the addition of another cohort to the replicate groups, stocking pressure on paddocks was increased. In 2008 each replicate group consisted of 12 or 13 animals, depending on which farm they were situated (Farm A or Farm B – see Table 2-1). High-nutrition animals grazed pasture from 2000 kg DM/ha on offer down to 500 kg DM/ha on offer, at which time they were moved to another paddock. A low-nutrition group then followed on to this paddock. Paddock area was halved during the spring using electrified tape in order to accommodate the increase in pasture growth rates and to limit the FOO available to the replicate groups. This must be considered when interpreting Table 2-3.

2.12.2 Grazing dry pasture

Pasture dried off in November of each year of the experiment. Animals were set-stocked on their paddocks at this time and measures of residual dry pasture and its quality were made in order to maintain the nutritional differential. Changing the amount of available feed was achieved by changing the area available for the animals to graze. The quality of the feed on offer was assessed fortnightly. As the season progressed and pasture dried and became less available, grazing area for both nutritional treatments was increased proportionately. When the feed quality or quantity reached a level where the high-nutrition treatment was potentially falling below maintenance levels, supplementary feeding was introduced. This occurred in mid January in each year.

2.12.3 Supplementary feeding

When residual dry pasture was no longer sufficient to feed the animals a supplementary feeding regimen was implemented. The supplementary feeding period began in January and finished in May in all years of the experiment. Calculations of nutritional requirements were made using GrazFeed 2.1 (CSIRO, Australia) which uses Feeding Standards for Australian Livestock Ruminants (SCA, 1990). At the start of the supplementary period calculations were made using the average liveweight for animals in each line and treatment. The high-nutrition diet was formulated to provide 1.2 times maintenance for energy and protein, while the low-nutrition diet was formulated to provide 0.8 times maintenance. This translated to a predicted growth rate of 0.5kg/day for the high-nutrition animals and a growth rate of -0.5kg/day for the low-nutrition animals during this time of year. The diets consisted of hay, harvested at VRC, and a formulated pellet supplement, Beef Pellets 825 (Wesfeeds, 31 Sevenoaks St., Bentley, Western Australia).

Hay was analysed and found to have the following nutritional content: DM % - 83.2, crude protein % - 8.37, DMD % - 67, ash % - 8, organic matter % - 92, organic matter digestibility % - 67, DMD % - 61, ME MJ ME/kg – 9.22, neutral detergent fibre - 53.15.

Pellets were also analysed and found to have the following nutritional content: DM % - 95, crude protein % - 20.9, DMD % - 72.6, ash % - 6.9, organic matter % - 93.1, organic matter digestibility % - 73.9, dry organic matter digestibility % -68.8, ME MJ ME/kg – 10.6, neutral detergent fibre – 42.2.

Hay was fed in round bales once or twice a week and pellets were fed every second day into long feeding troughs. Residue was estimated visually and recorded before the next feeding.

2.13 Blood parameter assays - hormones

Progesterone, GH, insulin, IGF-1 and leptin were assayed in this experiment. Assays were done at the Animal Science laboratory at the University of Western Australia.

2.13.1 Progesterone

Concentrations of progesterone in plasma samples were determined using an Active Progesterone Radioimmunoassay kit DSL 3900 (Diagnostic Systems Laboratories, Inc., Webster, TX) (Gray *et al*., 2000).

2.13.2 Growth hormone

GH standard

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Bethesda, MD, USA provided the oGH ("o" = "ovine") I-5. The stock solution (250 ng/ml) was stored in 0.05M phosphate buffer + 0.25% bovine serum albumin (BSA) at -20°C. One aliquot was thawed and diluted serially to the following concentrations: 0.49, 0.98, 1.95, 3.9, 7.8, 15.61, 31.25, 62.5 and 125 ng/ml in 0.05M phosphate buffer and 0.5% BSA on the day of assay.

Antiserum

Antiserum (NIDDK-anti-oGH-2) was provided by NIDDK. It was diluted 1:30,000 in 0.05M phosphate + 0.25% BSA + 1/700 NRS.

Iodination

GH was iodinated with $Na^{125}I$ using Chloramine-T method and purified with G100 Sephadex column. The reagents (5 µg growth hormone in 10 µL of 0.25M phosphate buffer, 0.5 mCi NaI^{125} in 5 µL, 5 µg Chloramine-T in 5 µL of 0.05M phosphate buffer) were placed in a reaction vessel and mixed for 30 seconds. The reaction was stopped by adding 2 µg of sodium metabisulphite in 10 µL of 0.05M phosphate buffer.

Following mixing, 250 µL of KI (0.05M phosphate buffer + 0.25% BSA + 0.1% KI) was added and the mixture transferred onto a 30 x 1 cm column of Sephadex 100. The reaction vessel was rinsed with 250 µL of KI which was then added to the column. Finally, the column was diluted with 30 ml of 0.05M phosphate buffer + 0.25% BSA and fractions were collected. The activity of the fractions was determined on a gamma counter, and the fraction after the protein peak was tested for the percentage of binding.

The fraction was diluted to a stock solution in 0.05M phosphate buffer + 0.25% BSA to give 250,000 cpm/50µL and stored at –20°C. On Day 1 of the assay, the tracer was thawed and diluted with 0.05M phosphate buffer, 0.25% BSA and 1/700 NRS to give 10,000 cpm/50µL.

Assay procedure

The assay included 6 replicates each of three quality control pools. On Day 1, plasma samples (100 µL), standards in 0.05M phosphate buffer and 0.25% BSA (100 µL) were diluted to 400 µL with 0.05M phosphate buffer and 50 µL of antiserum was added and incubated at 4°C overnight. On Day 2, 50 µL of tracer was added, centrifuged and incubated for a further 48 hours, after which time donkey anti-rabbit serum (50 µL: 1:7 in 0.05M phosphate buffer was added to all tubes before centrifugation at 1500g for 25 minutes at 4°C. The supernatant was decanted off and the activity of the precipitate was determined on a gamma counter (Downing *et al.*, 1995).

2.13.3 Insulin

Assay method

Insulin in plasma and was measured in duplicate by the double-antibody RIA method of Hales and Randle (1963) as modified by Bassett and Wallace (1966) and described by Tindal *et al.* (1978).

Buffers

Buffer 1, Phosphate buffered saline, consisted of 1 litre 0.1M stock phosphate buffer, 0.14 M sodium chloride (89g), 0.1% sodium azide (10g) and distilled water to 10 litres (pH 7.5).

0.1M Phosphate Buffer, stock phosphate buffer, consisted of 122.6g Na_2HPO_4, 21.2g $NaH_2PO_4.2H_2O$, and 10g sodium azide, dissolved to 10 litres double distilled water (DDW) (pH 7.5).

Insulin Standard

Highly purified crystalline ovine insulin (Sigma, Australia) was dissolved in dilute HCl, pH 2.5 to 3.0 to a concentration of 2 mg/ml. It was then diluted in 0.05M phosphate to 800µg/ml and stored in 10 µL aliquots at -20˚C and used for iodination. A 10 µL aliquot was diluted in Buffer 1 with 0.25% BSA to 200µU/ml, frozen and used as standards. On the day of the assay, after thawing, insulin was serially diluted to the following concentrations: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 µU/ml in Buffer 1 with 0.25% BSA.

Antiserum

Insulin antiserum (GP2, 21/7/80), was donated by Dr. Peter Wynn (CSIRO Division of Animal Production, NSW, Australia). It was raised in a guinea pig using bovine insulin (BI 4499, Ely Lilly Pty Ltd, Australia).

Iodination

Insulin was iodinated with NaI^{125} using chloramine T method and purified with G 100 Sephadex column. Eight µg of insulin in 10 µL 0.05M phosphate buffer, 20 µL 0.5 M phosphate buffer, 10 µL NaI^{125} and 20 µg chloramine T in 10 µL of 0.05M phosphate buffer were placed in a reaction vessel. The reaction was stopped 30 seconds later by adding 40 µg sodium metabisulphite in 50 µL of 0.05M phosphate buffer.

Following mixing, 200 µL of KI/ albumin/azide was added and the mixture was transferred into a 30 cm x 1 cm of Sephadex G100 column. The reaction vessel was rinsed with 200 µL of Buffer #4 which was then added to the column. The activity of the fractions was determined on a gamma counter, and the fraction after the peak was tested for percent binding and non-specific binding (NSB). The fraction was diluted to a stock solution in Buffer 1 with 0.25% BSA and 0.05m EDTA to give 250 000 cpm/50µL and stored at -20˚C. On Day 2 the tracer was thawed and diluted to give 10.000 cpm/100 µL.

Assay Procedure

The assay included 6 replicates each of three quality control pools. On Day 1, duplicate 100 µL plasma samples or standards were diluted to 200 µL with Buffer 1 and 0.25% BSA. Then 100 µL antiserum was added and the tubes were incubated at 4˚C overnight. On Day 2, 100 µL of the tracer was added, tubes were centrifuged and incubation was continued for 48 h at 4˚C. On Day 4, 100µL of goat anti-guinea pig serum (1:150 in Buffer #1 with 0.25% BSA) and 100µL of normal guinea pig serum was added and the tubes were centrifuged. After incubation overnight at 4˚C, 1.0 ml of 2% polyethylene glycol (PEG 6000) in Buffer 1 was added to the tubes before centrifugation at 1,500 x g for 25 min at 4˚C (Beckman, J-6M/E, USA). The supernatant was decanted and the pellets were left to dry overnight before the activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

2.13.4 Insulin-like growth factor-1

IGF-1 in plasma was measured in duplicate by the chloramine-T RIA method described by Gluckman *et al.* (1983). Interference by binding proteins was minimised by acid-ethanol cryoprecipitation method validated for ruminants by Breier *et al.* (1991).

Assay Buffer

The assay buffer consisted of Protamine sulphate 0.2 g/L, NaH2PO4.2H2O 4.68 g/L, EDTA 3.72 g/L, BSA 2.5 g/L and NaN3 0.2 g/L pH 7.5

Standard

Recombinant h (“h” = “human”) IGF-1 (Amersham Australia, North Ryde, NSW) was solubilised in 0.5M ammonium acetate, pH 5.5 at concentration of 1 mg/ml and stored at 20˚C. After thawing, IGF-1 was serially diluted in assay buffer to the following concentrations: .039, .078, .156, .312, .625, 1.25, 2.5, 5 and 10 ng/ml.

Antiserum

New Rabbit Antiserum to hIGF-1 (AFP4892898) was supplied by NIDDK. Dilute 1 in 10,000 for the assay.

Second antibody

Donkey anti-rabbit serum (DARS; 1:20) and NRS (1:500) was mixed to 1:5 DARS/NRS in the assay buffer.

Iodination of IGF-1

Recombinant hIGF-1 was iodinated using the chloramine T method, and purified with a pre-albuminated Sephadex G25 column and re-purified on a pre-albuminated 9 x 300 Sephadex G100 column (Pharmacia, Uppsala, Sweden).

5µg IGF-1 in 5 µL of 0.05 phosphate buffer, 50 µL 0.5M phosphate (no BSA), 10 µL NaI^{125} (1.0µCi) and 10 µL Chloramine-T (10 mg Chloramine-T in 10 ml 0.05M PO_4) were mixed in a vessel for 20 seconds and the reaction was stopped by adding 10 µL Na metabisulphite (3 mg Na-metabisulphite per ml in Buffer 1). The mixture was transferred onto a 30 cm x 1 cm column Sephadex G25. The reaction vessel was rinsed with Buffer 6 with pH adjusted to 6.2 which was then added to the column. Finally, the column was eluted with Buffer 6 and fractions were collected. The activity of the fractions was determined on a gamma counter, and the fractions at the protein peak were re-purified on a 9 x 300 mm column of Sephadex G100 fine eluted with Buffer 6. The activity of the fractions was determined on a gamma counter, and the fractions at the protein peak were collected and diluted to a stock solution in assay buffer 6 to give 250 000 cpm/100 µL and stored at -20°C. On Day 1 of the assay, the tracer was thawed and diluted 1:25 in Buffer 5 to give 10 000 cpm/100µL.

Assay procedure

On Day 1, 100 µL of plasma and quality controls were extracted by mixing with 400µL acid-ethanol (1:7 HCL: absolute ethanol) in glass tubes. The tubes were then vortexed and left to stand at 20˚C for 30 minutes before centrifugation at 1.500g for 30 minutes at 4˚C. The supernatant was decanted into plastic assay tubes and 0.855N Tris (base) was added to neutralise the solution (e.g., 250µL supernatant + 60µL Tris) before it was left to stand overnight at -20˚C. On Day 2 the samples were centrifuged at 1500g for 30 minutes at 4˚C (Beckman, J-6M/E, USA). The supernatant (100µL) was then added to 0.9 ml of assay buffer in new tubes. Then, 100 µL of the diluted samples were made to 300 µL with assay buffer and 100 µL of first antibody was added and incubated overnight at 4˚C. On Day 3, 100 µL of tracer was added, followed by incubation overnight at 4˚C, and on Day 4, 100 µL of second antibody/NRS mixture was added.

After incubation overnight at 4˚C, 1 ml of 6% polyethylene glycol (PEG 6000) in assay buffer was added, the samples centrifuged for 30 minutes at 1500 g, and the supernatant aspirated. The activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

2.13.5 Leptin

Leptin in plasma was assayed by double-antibody RIA in the same laboratory and as per the methodology described in Blache *et al.* (2000).

2.14 Blood parameter assays - metabolites

2.14.1 Beta-hydroxybutyrate

BHB was assayed using a Randox Kit (Randox Laboratories, Ltd, Antrim, U.K, Ranbut Catalogue Number RB1007) in an Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Tokyo, Japan) at the Department of Agriculture and Food, Western Australia.

2.14.2 Non-esterified Fatty Acids

NEFA was assayed using a Wako Kit (Wako Chemicals USA, Richmond, USA, Catalogue number 279-75401) in an Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Tokyo, Japan) at the laboratory of the Department of Agriculture and Food, Western Australia.

2.14.3 Glucose

Glucose was assayed using an Olympus kit (Olympus Optical Co. Ltd, Tokyo, Japan, Catalogue number OSR6121) in an Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Tokyo, Japan) at the laboratory of the Department of Agriculture and Food, Western Australia.

2.14.4 Acetate

Acetate was assayed using an Acetic Acid kit (Boehhringer Mannhiem, Germany, Catalogue Number 10148261035) on Roche Cobas Mira S Auto Analyser (F. Hoffmann-La Roche Ltd, Basel, Switzerland).

CHAPTER 3. MEASURING AND ASSESSING FATNESS, AND PREDICTING BEEF CARCASS RETAIL PRODUCT IN COWS SELECTED FOR A DIVERGENCE IN FATNESS OR FEED EFFICIENCY

3.1 Introduction

Fat is stored energy (Chilliard *et al.*, 1998a). The physiology of energy storage and retrieval in cattle is predominantly related to the processes of lipolysis and lipogenesis. Fat has a major role to play in cattle production systems because of this association with energy balance. Selection for fatness or leanness in cattle is possible (Crews, 2005; Liefers *et al.*, 2005; Upton, 2005), and the effects on aspects of the production system, particularly MP as defined earlier, remain largely undocumented. Fatness is related to body condition in cattle. BCS is a subjective measure of body composition in cattle (Randel, 1990; Tennant *et al.*, 2002) and has been shown to be an accurate and repeatable method of estimating body fat and energy reserves in beef cows (Wagner *et al.*, 1988).

Body condition in breeding females impacts on several areas of the production system. Body condition at the time of calving has been shown to be the most important factor affecting the postpartum interval (Richards *et al.*, 1986; Osoro and Wright, 1992; Wright *et al.*, 1992; Rhodes *et al.*, 2003) and BCS at parturition was also shown to affect birth and weaning weight of calves (Spitzer *et al.*, 1995).

Body condition is associated not only with traits that are related to productivity, but also with the concentrations of endocrine and biochemical parameters. Body fatness is the key factor that regulates adipose tissue expression of leptin as well as circulating plasma leptin concentrations (Frederich *et al.*, 1995; Blache *et al.*, 2000; Chilliard *et al.*, 2005). Fatness influences (Etherton and Bauman, 1998), and is influenced by (Etherton *et al.*, 1993) GH. NEFA and BHB are both products of fat mobilisation (Chilliard *et al.*, 1998a) and IGF-1 directly reflects an animal's energy status (Yelich *et al.*, 1995; Yelich *et al.*, 1996). These examples highlight the complex relationship between the fatness of an animal and its physiology as well as its fertility and level of production. In an experiment that is investigating the effect of selecting either for or against fatness, or of restricting energy supply, it is essential to understand how fatness affects MP.

The reason why leanness is a desirable trait in a beef production system is that it is associated with higher yielding carcasses (Wolcott *et al.*, 2001; Nkrumah *et al.*, 2004a). MP is broadly defined by inputs such as feed and outputs, predominantly Kg of beef sold (Lucy, 2004), whether it be weaners from the slaughter generation or culled cows. The number of kilograms of beef sold by a producer, and consequently the financial return, is directly influenced by the yield of beef in the carcass they produce.

There are also penalties for excess fat in a carcass (Egan *et al.*, 2001). Most processing facilities use grading systems which measure carcass fatness and price the product accordingly, with price penalties for carcasses with excess fat. The economic impetus for the selection of animals with lean carcasses is therefore significant. In this experiment the Genotypes of Fat and Lean were selected to provide a divergence in adiposity in the breeding animals, and therefore be representative of a divergence in lean meat yield in carcasses. However, it is not acceptable to assume that a divergence in fatness equates to a divergence in yield and for this reason the estimation of yield from measurements taken from live animals is a part of the experimental design.

It has been shown that weight and percentage beef carcass retail product (described as yield in kilograms) can be accurately predicted using US and live animal measures (Greiner *et al.*, 2003a). Using 534 steers these researchers developed and validated formulas to estimate carcass composition from live-animal US measurements. Measurements of final un-shrunk liveweight (liveweight), US 12th-rib fat thickness (Rib fat), US rump fat thickness (P8 fat) and US longissimus muscles area (EMA) taken five days before slaughter were used in a regression equation to predict the weight of totally trimmed, boneless retail product and lean trim adjusted to 20% fat from one carcass side (yield). It was shown that 83% of the variance in yield can be accounted for using their published equation. This research concluded that live animal measurements can be reflective of potential yield in beef cattle and this methodology has been used in the current experiment to predict yield in the experimental animals.

This chapter is an overview of the adiposity changes and carcass yield (yield) predictions in the experimental animals (see section Chapter 2). It includes the methodology used to obtain fat measures, the results and some discussion of these results. It was a major hypothesis of this thesis that nutrition rather than Genotype influences the productivity of the cows in this experiment. However, because the different Genotypes have been shown to be associated with differing levels of body fat, it was necessary to quantify fatness in the animals in the current experiment to test the hypothesis. For this reason the adiposity of the cows during the breeding seasons of 2007 and 2008 has been described in this chapter and the interaction between fatness and the main experimental treatments of Genotype and nutrition has been explored. Subsequent chapters frequently refer to the results and conclusions contained within this chapter.

3.2 Aims

1. To validate the US measures obtained by the author compared to those obtained by an accredited scanner.
2. To measure and analyse fatness in the experimental animals pre-calving and during the breeding season, and to evaluate the change in fatness over this time.

3. To predict yield using measurements taken from and live animals and analyse these results.

3.3 Hypotheses

1. There would be a strong relationship between US measures taken by the author and those taken by the accredited scanner.
2. Fat and high-NFI animals would be fatter than Lean and low-NFI animals during all measurement periods.
3. Nutritional treatment would impact on fatness with animals on high-nutrition being fatter than those on low-nutrition.
4. Lean and low-NFI animals would have higher predicted yields than Fat and high-NFI animals.

3.4 Materials and methods

The methods used to obtain ultrasound measures of P8 fat depth are described in Section 2.8. The author (A) was responsible for the majority of the scans for the subcutaneous fat measures but an accredited scanner (AS) was responsible for a bi-annual full herd scan. The scan by the accredited scanner included measurements of EMA and IMF. Accredited scanners are able to obtain US measures of P8 fat in a few seconds which makes the technique efficient and non-invasive. In Australia and New Zealand, scanners need to pass a proficiency test to become and remain accredited scanners. Breedplan only accepts scan data obtained by accredited scanners. The proficiency test is conducted by the Performance Beef Breeders Association (PBBA). The PBBA Secretariat is currently in the care of Australian Limousin Breeders Society Ltd, PO Box 262, Armidale, NSW 2350. They evaluate the ability of beef cattle scanners to obtain repeatable scan measures on live animals and accurately to estimate the actual carcass trait. Scanners are required to be re-accredited every three years.

All experimental animals had US measures of fatness taken during the breeding season. In 2007 there was a single scan in May, about one month before the start of calving. After calving began there was a two week period in the middle of the calving period when all animals were scanned. The animals were then scanned again at the end of the joining period.

To validate the results of the author’s technique a full scan was performed by the author 20 days before a full herd scan performed by the accredited scanner. A shorter time interval between scans was not logistically possible. One hundred and ninety five animals were scanned on each occasion and the results obtained by the two scanners were compared. Results are detailed in section 3.5.

Measurements were taken more frequently in 2008. The first pre-calving scan was done in April, about one month before calving began in May, and thereafter scans were conducted monthly from May to December. The covariate *3^{rd} trimester P8*, used in analytical models, refers to the scans taken before calving began, and as the name suggests is designed to be a reflection of adiposity in the last trimester of the animals’ pregnancy. Measurements of P8 fat depth, rib fat depth (measured between the 12^{th} and 13^{th} rib), EMA and BW were taken at the end of the calving season in 2008 by the accredited scanner. These results were used to predict yield in the animals. Regression equations, published by Greiner *et al.* (2003a), shown in Table 3-1, were used to predict yield in the experimental animals. The equation delivers results on a half-carcass basis and before analysis the results were multiplied by two to represent whole-carcass predictions.

Table 3-1: Prediction equation for weight and percentage of retail product (yield) developed from live animal measurements adapted from Greiner *et al.* (2003a).

dependent variable and equation	R^2	RMSE	Cp	Partial Regression Coefficient Intercept	Rib Fat cm	P8 Fat cm	EMA cm2	Liveweight
Yield	0.83	5.3	4	-5.39	-8.597	-5.17	0.437	0.161

3.4.1 Statistical analysis

For a general description of statistical analyses and an explanation of fixed and random terms in the models described below see Section 2.10. P8 data was log transformed for all analyses in this chapter.

3rd trimester P8 fatness

P8 fatness in the last trimester of pregnancy was analysed to identify significant effects of Genotype (line, FatvsLean, HiNFIvsLoNFI) and nutrition and their interactions on P8 fatness during that period. The LMM had the following fixed and random models:

Fixed model:

constant + location + cohort (2008 analysis only) + line + height + FatvsLean + HiNFIvsLoNFI + calving date + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition

Random model:

replicate group + dam ident

Note that the order of terms in the fixed model indicates the order of hierarchical testing. For instance, Comparisons between Fat and Lean animals and between high-NFI and low-NFI animals were tested after removing the effect of height, whereas the effect of line was not adjusted for height because it is believed that the height difference between industry and NFI animals is part of the Genotype.

Predicted means for each Genotype were adjusted for location and cohort but adjustments for height were only made within line and no adjustment was made for calving date. The decision to adjust for a particular covariate was made on the basis of whether the covariate was seen as an inherent element of a Genotype or not.

P8 fatness during the breeding season 2008

P8 fatness across the breeding period (up to 80 days pre-calving until the end of joining) was analysed to identify significant effects of Genotype (line, Fat vs. Lean, HiNFIvsLoNFI), nutrition and their interactions on P8 fatness during that period. Only the results for the 2008 breeding season were analysed because US measures of subcutaneous fat were made far less frequently during the 2007 season.

P8 values were log transformed prior to analysis. The LMM had the following fixed and random models:

Fixed model:

constant + location + cohort (2008 analysis only) + lactating + calving date + line + height + FatvsLean + HiNFIvsLoNFI + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition + days-post-calving + days-post-calving.nutrition + days-post-calving.line + days-post-calving.FatvsLean + days-post-calving.HiNFIvsLoNFI + days-post-calving.line.nutrition + days-post-calving.FatvsLean.nutrition + days-post-calving.HiNFIvsLoNFI.nutrition

Random model:

replicate group + replicate group.days-post-calving + dam ident + days-post-calving.dam ident

Since the term days-post-calving is a variate this model assumes a linear response in P8 to *days-post-calving*. The model is sometimes referred to as a random coefficient model as the linear *days-post-calving* coefficients for each dam were part of the random model.

Change in P8 fatness during the breeding season

The change in fatness of the animals between calving and the end of joining was estimated and analysed. A regression analysis was fitted using a third trimester measure of P8 fat and then all measures of P8 fat from the time of calving until the end of joining. This produced a regression coefficient (P8 slope) for each animal. This slope then became a y-variate which could be fitted using a LMM.

Fixed model:

constant + location + cohort (2008 analysis only) + line + height + FatvsLean + HiNFIvsLoNFI + calving date + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition

Random model:

replicate group + dam ident

Predicted yield and liveweight change over the breeding cycle.

Predicted yield results for each animal, as well as a calculation of liveweight change of the breeding cycle (difference in liveweight (kg) from weaning to weaning) were analysed using linear mixed models.

Fixed model:

constant + location + cohort + calving date + line + FatvsLean + HiNFIvsLoNFI + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition

Random model:

replicate + dam ident

Appendix 9.6 shows the level of significance (P-values) for all statistical models used in this thesis.

3.5 Results

3.5.1 Comparison of scan results obtained by the author and an accredited scanner

The comparison between the author's results and those obtained by the accredited scanner has been made using US measures taken 20 days apart.

Figure 3-1 illustrates that the author measures are on average higher than the accredited scanner measures for the same animal. While the difference is negligible for low fat scores it becomes bigger as fat score increases. There is clearly a relationship between the two fat scores but since deviations from the regression line will increase with fat score it was necessary to transform the fat scores before fitting a regression line.

In Figure 3-1, P8 measurements have been transformed to a logarithmic scale, $\log_e(x+5)$ and a linear regression has been fitted. The linear regression equation was:

$$\log_e (AS+5) = 0.04407\ (\pm 0.079) + 0.8043(\pm 0.026)*\log_e (A+5)$$

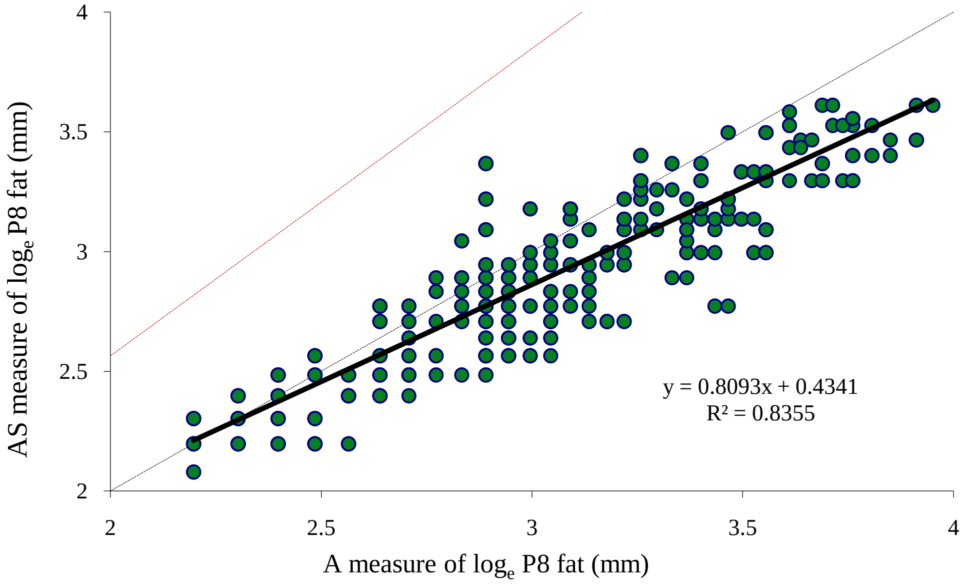


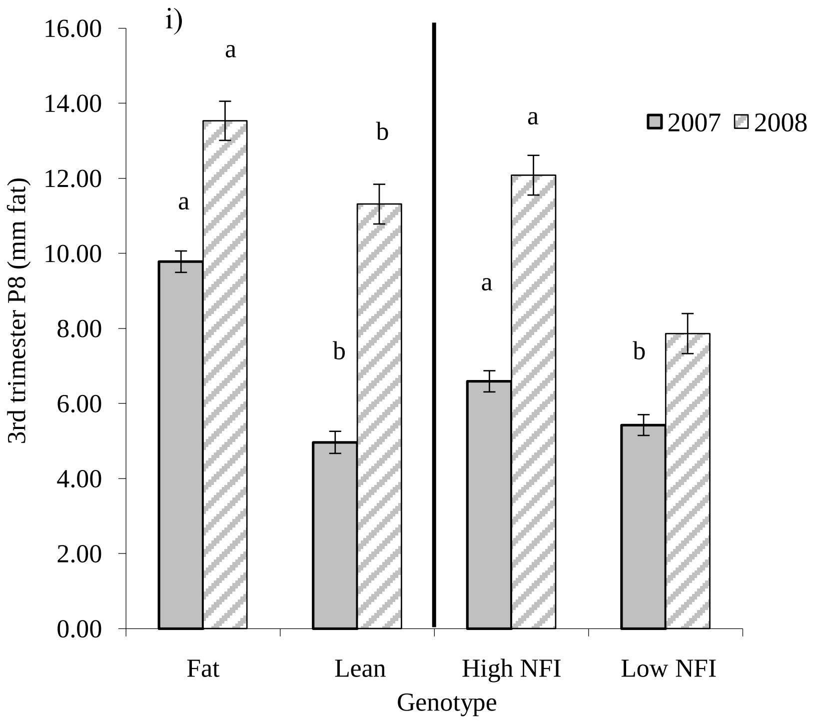
Figure 3-1: Scatter plot of $\log_e$ P8 data (A vs. AS) with a one-to-one (i.e. x=y) red dashed line drawn on the same graph. Regression line (solid line) formula included

The adjusted R^2 was 0.829, which suggests that 83% of the variance in accredited scanner measures could be predicted by using measures taken by the author to predict accredited scanner measures based on the regression line. Similarity of scores at the low end suggests that differences at the high end were less likely to be due to change in fat over the three week difference, and more likely to be due to a difference in scoring. SE suggest that 95% of points in graph are within ± 2 standard errors. A significant proportion of variance was explained by the regression ($F < 0.001$), meaning that the chance of accounting for this much variance when there is no real relationship between operators' measures is very small. The regression coefficient for the intercept is significantly greater than 0 ($P < 0.001$). The coefficient for the *X* variable (0.804) is significantly less than 1.

The analysis shows that the two operators had scores that had a highly significant relationship. The results obtained by the author can therefore be assumed to be accurate measures of carcass fat depth.

3.5.2 3rd trimester P8 fatness

In both years in their third trimester of pregnancy Fat animals had higher US measures of P8 than Lean animals (2007: $P<0.001$; 2008: $P = 0.01$) and high-NFI had higher US measures of P8 than low-NFI animals (2007: $P = 0.065$; 2008: $P<0.001$). In 2008 animals on high-nutrition had higher US measures of P8 ($P < 0.001$) than those on low-nutrition (see Figure 3-2). Nutrition means were adjusted for all covariates. Cohort affected the amount of US measured fat pre-calving ($P<0.001$), where older animals (1st cohort) had higher US measures of P8 than younger animals (2nd cohort – results not displayed). Significance values (P-values) and LMM model structure used for the analysis of 3rd trimester P8 is shown in Table 9-2.



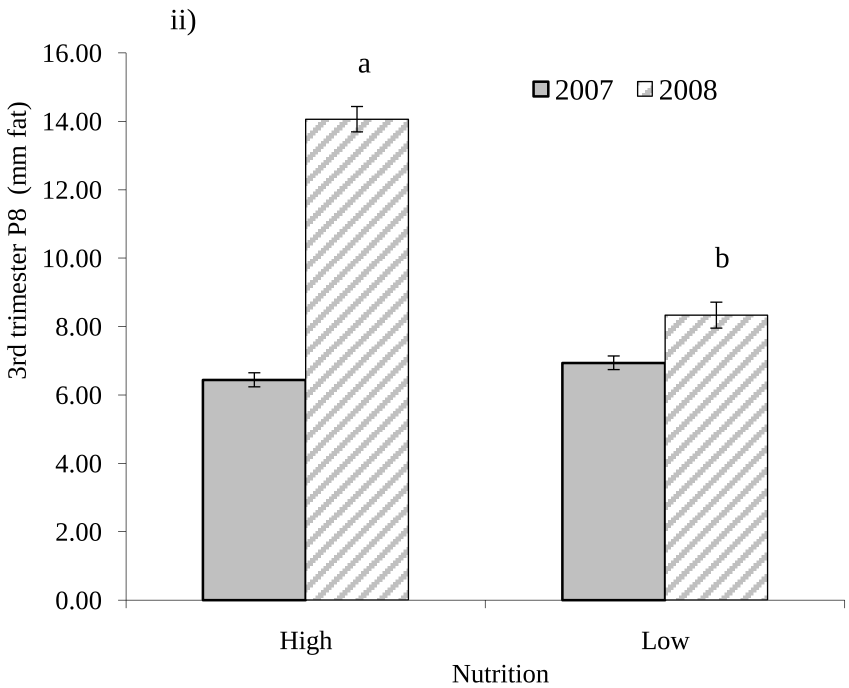


Figure 3-2: Main effect of i) Genotype and ii) nutrition on 3rd trimester US measures of P8 (mm) in 2007 and 2008. Within line and year, means with different letters differ significantly (P < 0.05). Error bars represent 68% confidence intervals.

3.5.3 P8 fatness during the breeding season 2008

The analysis of P8 from the pre-calving period through to the end of joining showed that both Genotype and nutrition affected fatness. Figure 3-3 shows the individual US P8 measures for cows within each replicate group during the breeding season in 2008. On this figure replicate groups are annotated as “Plot” with the suffix defining Genotype, nutritional treatment and replicate group number. For example, “BL2” refers to animals in the Fat genotype on the low-nutrition treatment in replicate group 2. “O” represents high-NFI, “W” represents lean and “P” represents low-NFI.

There was no indication of a curvilinear trend over time to the results; rather there was on average a linear increase in US P8 fat from the start to the end of the breeding season with some large fluctuations of P8 measures at different times that were different between cows. A linear effect of days-post-calving in the random coefficient model (Section 3.4.1) was therefore justified.

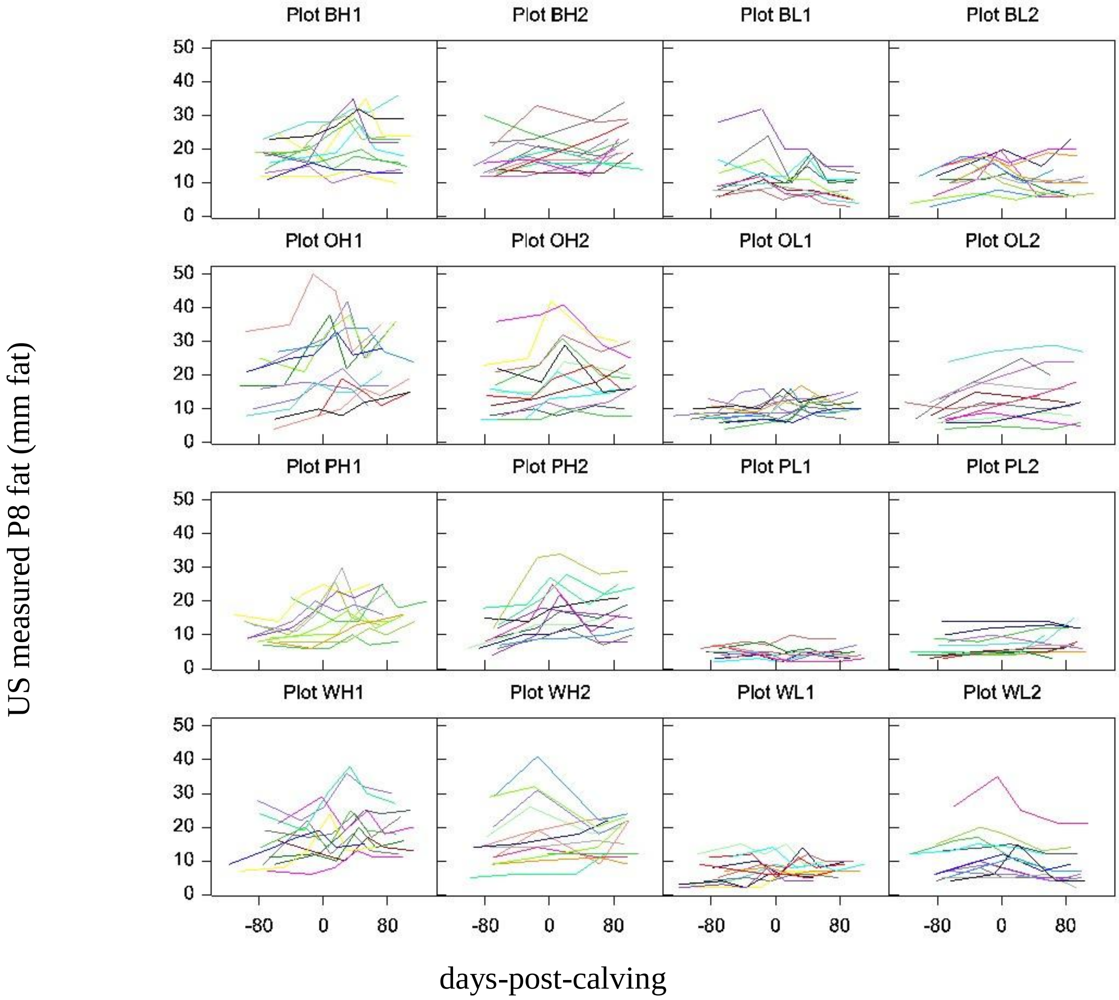


Figure 3-3: Trellis plot of individual cow P8 measures (mm fat), within replicate groups, during the breeding season in 2008.

Fat animals had higher US measures of P8 fat than Lean animals (P = 0.044, Table 9-3) and high-NFI animals had higher US measures of P8 fat than low-NFI animals (P = 0.003, see Figure 3-4, Table 9-3 pg 312). Animals on high-nutrition had higher US measures of P8 fat (P < 0.001,) than animals on low-nutrition and the 1st cohort had higher US measures of P8 fat than the 2nd cohort (P < 0.001). Non-lactating animals had higher US measures of P8 fat than lactating animals ((P = 0.041, see Figure 3-4). There were no interactions between the main effects of Genotype and nutrition. As the number of days-post-calving increased animals had higher US measures of P8 fat (P = 0.003) but NFI animals gained fat at a rate of 1mm/day faster than Industry animals (P = 0.026). Significance values (P-values) and LMM model structure used for the analysis of P8 fatness during the breeding season in 2008 shown in Table 9-1.

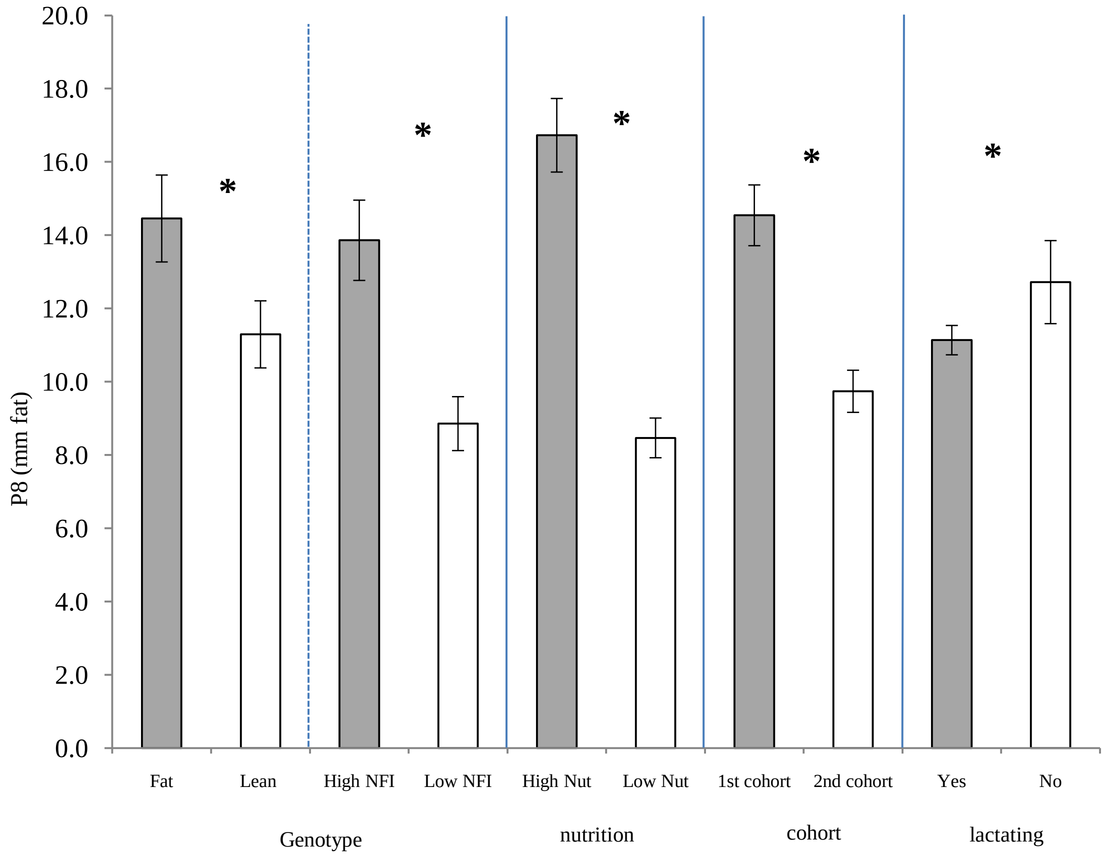


Figure 3-4: Mean P8 fat (mm fat) for categories Genotype (Fat, Lean, high-NFI, low-NFI), nutrition (high-nut, low-nut), cohort (1st cohort, 2nd cohort), lactating status (yes, no) in 2008. Within category, * denotes means differ significantly (P < 0.05). Error bars represent 67% confidence intervals.

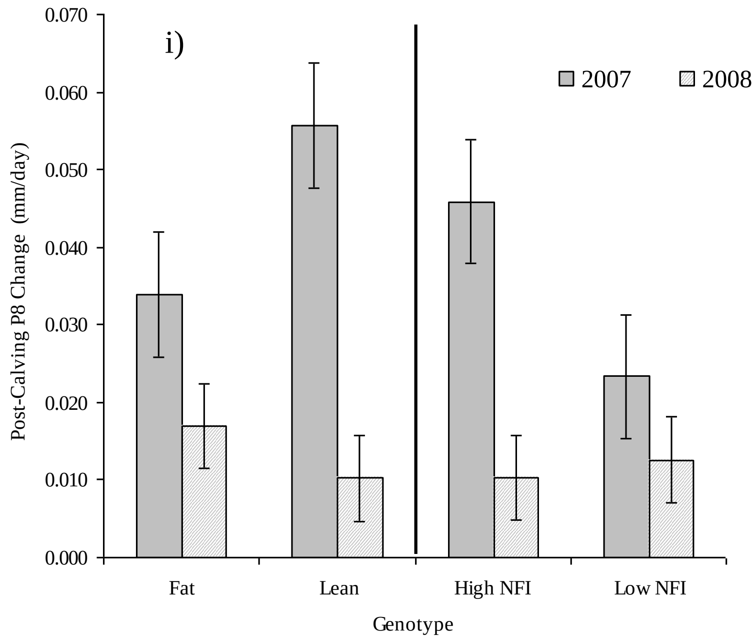
3.5.4 Change in P8 fatness during the breeding season

Animals on high-nutrition accrued fat faster than those on low-nutrition ((2007: P = 0.009; 2008: P = 0.006, see Figure 3-5). Genotype within line, however, had no effect on the rate of change of fatness post-calving. The rate of fat accretion was different for each year. In 2007 the animals accrued fat faster than in 2008 (see Figure 3-5).

Height (P = 0.006) and calving date (P = 0.037) affected the rate of fat accretion, with taller animals having a slower rate of fat accretion and the rate of accretion slowing as time progressed after calving (see Table 6-1). Older animals (1st cohort) accrued fat more slowly (P = 0.003) than younger animals (2nd cohort).

Chapter 6 addresses the relationships that exist between fatness and various blood metabolites. Part of this analysis was to examine how the rate of change of fatness is associated with various hormones and metabolites. The rate of fat accretion was significantly correlated with post-calving BHB (r = -0.3108) and leptin (r = 0.1812): see Table 6-5. The rate of fat accretion was also significantly correlated with the rate of change of acetate (r = -0.150), BHB (r = 0.177) and leptin (r = 0.158) post-calving (see Table 6-6).

Significance values (P-values) and LMM model structure used for the analysis of the change in P8 fatness during the breeding season in 2008 is shown in Table 9-2.



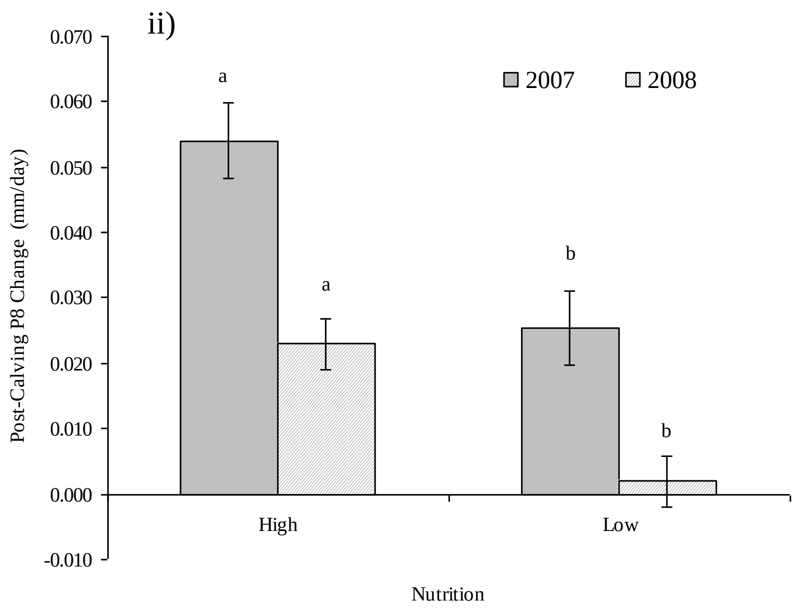


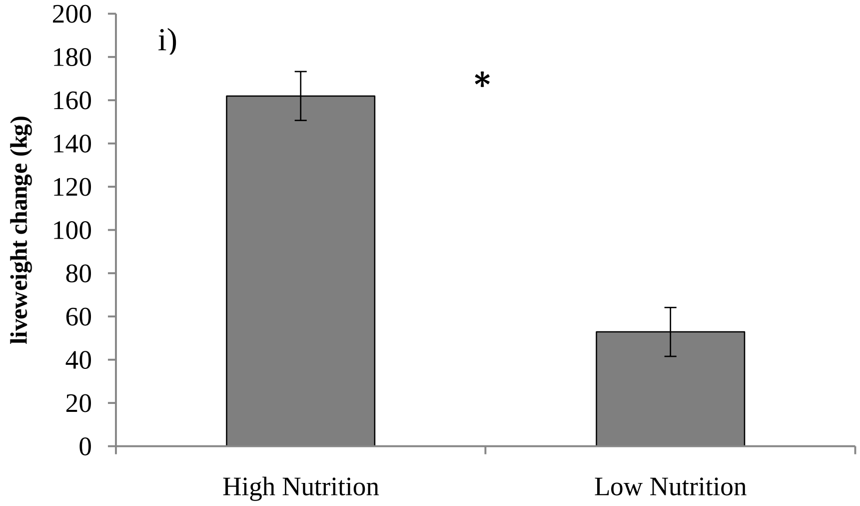
Figure 3-5 Mean rates of fat accretion (mm P8 fat/day) post-calving in 2007 and 2008 for each i) Genotype and each ii) nutritional treatment. Within line and year, means with different letters differ significantly (P<0.005). Error bars represent standard errors.

3.5.5 Predicted carcass yield and liveweight change over the breeding cycle

Lean cows had higher predictions of yield than Fat cows (P =0.028) and low-NFI cows had higher predictions of yield than high-NFI cows (P =0.050; see and Figure 3-7). Cows on low-nutrition had lower (P<0.001) predicted yields than those on high-nutrition (see Figure 3-6). Younger cows (2^{nd} cohort, P <0.001) had lower predicted yields than older cows (1^{st} cohort - results not presented).

Cows on low-nutrition had a lower (P < 0.001) change in liveweight over the breeding season in 2008 than cows on high-nutrition (see Figure 3-6). Liveweight change increased as calving date became closer to the end of the calving period (P < 0.001, 0.53kg/day ± 0.071).

Significance values (P-values) and LMM model structure used for the analysis of predicted yield and liveweight change over the breeding season cycle is shown in Table 9-3.



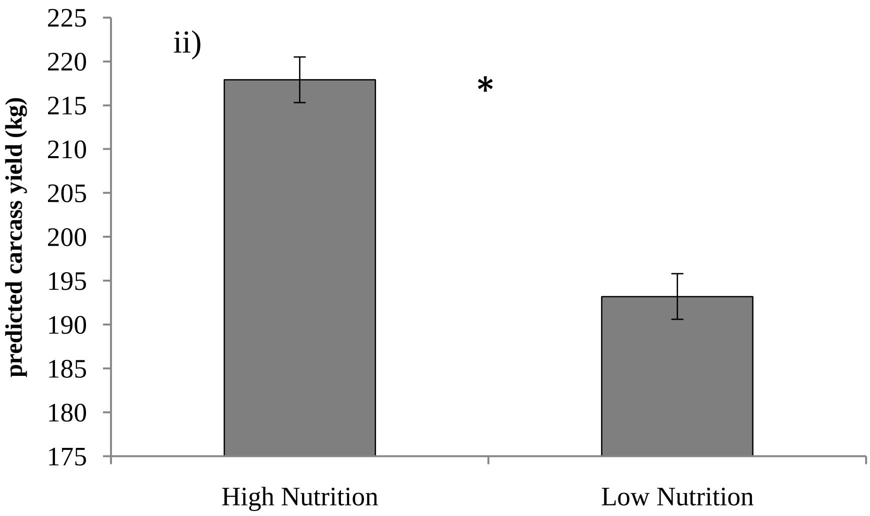


Figure 3-6: nutrition effect on i) Liveweight change over breeding season 2008 and ii) Predicted carcass yield (kg). * denotes means differ significantly (P <0.001). Error bars represent standard errors.

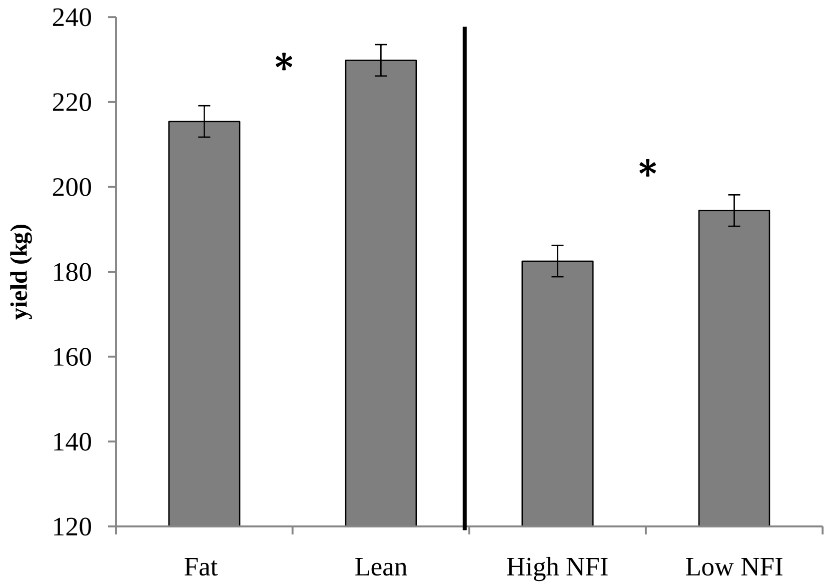


Figure 3-7: Genotype (Fat vs. Lean, high-NFI vs. low-NFI) means for predicted carcass yield (kg). Within line, * denotes means differ significantly (P <0.001). Error bars represent standard errors.

3.6 Discussion

US measures of P8 fat in live animals are good indictors of adiposity and in combination with other US measures are good predictors of retail beef yield (RBY). US measures of P8 fat depth, taken at slaughter, were the most useful predictor of RBY, accounting for 52% of the variation in RBY (Wolcott *et al.*, 2001). Greiner *et al.* (2003b) reported an overall correlation of 0.89 between US measures of backfat at the P8 site and carcass fatness. These conclusions, along with confidence in the accuracy of the use of US to measure fatness, are supported in other literature (Brethour, 1992; Hamlin *et al.*, 1995; Wall *et al.*, 2004; Schroder and Staufenbiel, 2006). In this experiment animals were measured by the author as discussed and illustrated in Section 2.8. Comparison of the measurements taken by the accredited scanner and the author respectively (see Section 3.5.1) reveal that there was a significant positive relationship between them. The linear regression between the measurements had an R_a^2 of 0.83 ($P < 0.001$) indicating that measurements made by the author can be used to predict those for the accredited scanner and that measurements obtained by the author (these being the majority of the P8 measurements obtained during the breeding season) can be used to compare body fatness in the animals. In this discussion animals with higher US measure of P8 fat are considered "fatter" than those with lower US measures of P8 fat. There was some difference between scanners and part of this could be attributed to the time difference between the authors and the accredited scanners measurements.

The key finding in the results from the analysis of pre-calving P8, P8 fatness across the breeding season and P8 change post-calving, was that both Genotype and nutrition impact upon P8 fatness but the effects did not interact. Fat animals were fatter than Lean animals, high-NFI animals were fatter than low-NFI animals, and animals on high-nutrition were fatter than those on low-nutrition throughout the experiment. This result differs from results published by Herd *et al.* (1998) who reported that high-NFI cows grazing pasture were lighter in weight, but no fatter, than low-NFI cows. They concluded that this could imply an association of efficiency with maturity pattern. However, several other studies concur with our results that high-NFI cattle are fatter than low-NFI cattle (Davis and Simmen, 2000; Herd and Bishop, 2000; Richardson *et al.*, 2001; Johnston *et al.*, 2002; Crews, 2005).

There was no nutritional effect on pre-calving fatness in 2007. The reason for this was that allocation to experimental treatments occurred in May 2007, too soon for the treatment effect to be reflected in difference in fatness. The other exception was that the rate of fat accretion was the same for all Genotypes within lines in 2008. In 2008 the rate of change was very significantly influenced by nutritional treatment with high-nutrition animals accruing fat faster than low-nutrition animals. It is probable that the effect of the nutritional treatment overshadowed the Genotype effect.

Fatness is a product of the balance between energy supply and demand, as well as a genetic predisposition to fatness or leanness. Fat animals on the same level of nutrition were uniformly fatter than Lean animals, confirming that the selection criterion used to select these animals, namely a difference in EBV for fatness, is a powerful and effective tool for producers to use to select and breed animals with a particular phenotype. This was especially notable in that the animals selected for a divergence in fatness based on the rib fat EBV were selected purely on the basis of the parental average EBV, a breeding value that is calculated without any of the particular animals' phenotypic results. Similarly, the differences in fatness previously identified in animals of varying net feed efficiency (Davis and Simmen, 2000; Herd and Bishop, 2000; Arthur *et al.*, 2001; Johnston *et al.*, 2002; Nkrumah *et al.*, 2004a; Arthur *et al.*, 2005; Crews, 2005) were notable in the VRC experiment animals, and these differences were present throughout the breeding season.

All factors that influence the energy demand on the cow have an impact on fatness. Nutrition, lactation, age (cohort), calving date, days-post-calving and height all had significant effects on P8 fat depth. Nutritional treatments resulted in a difference in energy supply with energy restricted in low-nutrition treatments. Lactating cows have a higher energy demand than non-lactating cows and young cattle have the added energy requirements of growth (see Figure 3-4). Hence fatness was lower in all of these categories in the experiment. Animals that calved later in the breeding season had access to higher planes of nutrition because of the increased pasture availability as the season progressed. As time after an animal's calving date (days-post-calving) increased energy supply improved through increased pasture availability and nutritive quality and the reducing burden of lactation. Therefore calving date and the number of days-post-calving had a significant positive effect on P8 fatness.

Consistent with published literature (Wolcott *et al.*, 2001; Nkrumah *et al.*, 2004a; Rourke *et al.*, 2009), the results of the current experiment show that fatter cows had lower predicted carcass yields. It is concluded that because fatness is lower in more feed-efficient and Lean cows, they deliver the benefit of potentially using fewer MJ ME to produce fat and so consume less feed with the benefit of carcass yield. It has been shown that consumers are prepared to pay more for meat that meets particular market specifications and this includes leaner meat (Polkinghorne, 2006). As a consequence processing facilities often have a price penalty for animals with too much fat on the dressed carcass. The other important, industry-relevant outcome of this analysis is that selection for reduced fatness using the EBV for rib fat is a useful tool in increasing profitability through the association of leanness with increased yield. This confirms the premise on which the experiment is based, namely that selection for leanness is beneficial to the beef producer, both because of the potential reduction in feed input as well as the more profitable output in terms of kg of beef sold. It is recognised that the major output in a beef enterprise is the slaughter generation whose yield is not tested in this experiment, but it is feasible to suggest that to use a bull with favourable fatness EBVs and cows selected for leanness would result in progeny with similarly favourable carcass yields. It is argued that the experimental design closely mimics industry practice where a producer has selection of leaner cattle as a priority in their enterprise.

Liveweight change over the breeding period is a measure of both an input and an output in the production system. Growing animals, or animals putting on weight, require the input of feed but the liveweight is translated to an output when the animal is sold at the end of its breeding life. It was notable that there was no difference in liveweight change between the Genotypes over the breeding cycle so the output in terms of culled cow meat yield would be the same across all Genotypes. Nutrition was a significant main effect as discussed but this is to be expected and the effect was the same across all

Genotypes. As a consequence the change in liveweight over the breeding cycle is not considered further in the analysis of the efficiency of the production system, in particular in Chapter 5 where measures of the efficiency of the beef production system were considered.

This chapter outlined only the associations between Genotype and nutrition and fatness in the experimental cattle and the predictions of yield for the different experimental Genotypes. The described results and changes in this chapter will be discussed in subsequent chapters in the context of their relationship with the particular aspects of MP addressed in those chapters.

CHAPTER 4. ESTIMATION OF THE LENGTH OF THE POST-PARTUM ANOESTRUS INTERVAL IN BEEF COWS ON AN EXTENSIVE GRAZING SYSTEM

4.1 Introduction

A key indicator of productivity in cattle is the length of the post-partum anoestrus interval (PPAI), defined as the period between parturition and the resumption of oestrous activity (Montiel and Ahuja, 2005). In a beef cow/calf production system in the South West of Western Australia most producers adhere to a seasonal calving calendar whereby cows are joined, generally for a period of between six and nine weeks, approximately six weeks after the end of the calving season. The aim is to achieve conception within 85 days following parturition so that calving-to-calving intervals are maintained at 365 days (Crowe, 2008). Any cow that has not resumed its reproductive cycling by the end of the joining period will become a non-productive animal and be culled from the herd. This increases the annual rate of replacement heifer purchase. Even a prolonged PPAI can lead to an increase in days-to-calving which reduces productivity over the lifetime of the cow. Across the herd it also extends the calving distribution with long joining seasons required to achieve adequate conception rates. A recognised way to assess productivity therefore is to get some measure of the length of the PPAI (Reist *et al.*, 2000; Crowe, 2008; Konigsson *et al.*, 2008). Several methods are used, particularly in dairy cows, (Diskin and Sreenan, 2000), but in beef cows the options are fewer because of the difficulty in using methods which require intense observation or frequent handling. There are, however, two methods that possibly suit a beef production system. The first is the use of a device that highlights physical manifestations of oestrus, or oestrous behaviour, such as a pressure sensitive device mounted on the back of each cow which can be triggered when the cow stands for mounting (Foote, 1975). The efficiency of using these pressure sensitive devices, such as Kamar® Heatmount® Detectors and Estrus Alerts™, is variable from 44-96% from studies in dairy herds (Diskin and Sreenan, 2000).

The second method is to use an endocrine measure of ovarian activity such as blood progesterone. It has been reported that plasma progesterone concentrations post-calving remain between 0.1 and 0.3ng/ml until the first ovulation when the cow enters the luteal phase. Progesterone concentrations of >1ng/ml are indicative of an ovulation having occurred (Echternkamp and Hansel, 1973). Garmo *et al.* (2009) used progesterone concentrations to estimate the resumption of oestrus post-calving and defined the commencement of luteal activity (indicating that a cow has ovulated) as the first day of 2 consecutive measurements of progesterone concentration ≥3 ng/mL not earlier than 10 days after calving. In another study it was concluded that cows with progesterone concentrations >7 ng/mL were pregnant (Garmo *et al.*, 2008). It has been shown that it is possible to develop a profile of the ovarian activity by performing radio-immunoassay of progesterone in milkfat of samples taken twice weekly (Opsomer *et al.*, 1998).

This chapter is written to quantify the PPAI in the experimental cattle so that this result can be used in the analysis of MP in subsequent chapters. It is "stand alone" for ease of reading and to include some discussion specific to the estimation of the PPAI in the cows.

4.2 Aim

1. To use post-calving progesterone profiles to quantify the PPAI in beef cows on an extensive grazing production system.

4.3 Hypothesis

That fortnightly measuring of blood progesterone would not be frequent enough to give accurate estimates of PPAI.

4.4 Materials and methods

4.4.1 Animals

The animals used in this experiment are as described in Chapter 2 (VRC experimental animals). Table 2-1 in that chapter outlines the experimental design and allocation of animals to the experiment. Because in 2007 only the 1st cohort of animals had calved, the data in that year was sourced from the 1st cohort only, whilst in 2008 both cohorts were included in the data set.

4.4.2 Data collection

Animal data

Blood samples were collected from all animals in the last trimester of pregnancy and then during the post-calving period, as described in Section 2.6. One blood sample was taken in the last trimester of pregnancy and multiple samples were taken post-calving as per Section 2.9.3.

Measurement of fatness

All experimental animals had US measurements of fatness taken during a two-week period in the middle of the calving period in 2007 as per Section 2.8. Measurements were taken more frequently in 2008 where US scans were conducted monthly from May to December. This period included the 3rd trimester of pregnancy until the end of the joining season.

Pasture data

Pasture data was collected during the growing season as described as per Section 5.4.3.

4.4.3 Estimation of post-partum anoestrus interval

In both calving seasons an attempt was made to estimate when ovarian activity recommenced post-calving and gain an impression of the length of the PPAI. Because of the experimental design, animals were spread over a large area of land in small groups and this made some of the recognised methods of oestrus detection, such as intensive herd observation, impossible. No visual observation of oestrous behaviour, such as mounting, was attempted. Therefore, only two methods of oestrus detection were used in this experiment. The first was to use a device that highlighted physical manifestations of oestrus, or oestrous behaviour, such as a pressure sensitive device mounted on the back of each cow which can be triggered when the cow stands for mounting (Foote, 1975). Estrus Alert, © Western Point Inc, USA Patent #6, 467,430, a heat detection system applied to the tail head by brushing the hair, warming the Estrus Alert and sticking it firmly to the tail head, was used in this experiment. The stickers were read by examining the extent to which the shiny surface had been removed. This occured when the cow wearing the alert was mounted by another, an indication the mounted cow was showing behavioural oestrus. The Estrus Alerts were applied to the cows the first time they were mustered after calving. The problem encountered was that the inclement weather during the Western Australian winter, and animals' habit of rolling in the dirt and mud, meant that most of the Estrus Alerts were either rubbed loose from the skin or were washed off during the post-calving season. As the groups of animals were handled only once every two weeks it was impossible to do any "running repairs" on removed or damaged alerts, and because it was only during this fortnightly mustering that observations were recorded, it was decided that inadequate and unreliable information was being obtained via this method. Scientific interpretation of the rubbings was deemed impossible and the use of Estrus Alerts was abandoned after the 2007 breeding season. As a consequence it was decided in 2008 to try a different method of estimating return to oestrus.

The second method used was an endocrine measure of ovarian activity, specifically the measurement of blood progesterone. Methodology for the collection of blood samples to use in the measurement of progesterone was the same as that described in Section 2.6. Analysis of progesterone concentration in serum was performed as described in Section 2.13.1. Increases in serum progesterone were used to identify the time to first post partum ovulation and the aim was to use this information to estimate the length of the PPAI.

An increase in progesterone of >2 ng/ml from the first available sample (the "baseline" level – usually approximately two weeks post-calving) was defined as an elevated concentration and was used to identify an animal that was in the luteal phase and therefore had ovulated. Estimation of the timing of the ovulation was limited to sometime between when the elevated measure was taken and the preceding measure. For the purpose of comparing genotypes and nutrition treatments, a figure defined by the number of days-post-calving on which an elevated measure of progesterone was taken, was allocated to the animal as d*ays to first ovulation*.

The results of a statistical analysis of *days to first ovulation* as well as the result of the days-to-calving analysis from section 5.5.1 were used together to identify difference in PPAI in different Genotypes or at different levels of nutrition.

4.4.4 Statistical Analysis

For a general description of statistical analyses and an explanation of Fixed and Random terms in the models described below see Section 2.10.

A LMM with the following fixed and random models was fitted to the data in order to examine the effects of Genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition and their interaction on days to first ovulation:

Fixed model :

constant + *location* + *cohort (2008 analysis only)* + *line* + *FatvsLean* + *HiNFIvsLoNFI* + *calving date* + *3rd trimester P8* + *nutrition* + *line.nutrition* + *FatvsLean.nutrition* + *HiNFIvsLoNFI.nutrition*

Random model:

replicate group + *dam Ident*

In the fixed model 3rd trimester P8 is included as a covariate which is likely to have an effect on days to first ovulation. This covariate might be regarded as part of the genotypes, so genotype effects are not adjusted for 3rd trimester P8 while the effect of nutrition is adjusted.

4.5 Results

The Estrus Alert method of detecting resumption of oestrus, and therefore the length of the PPAI, that was used in 2007 was deemed to yield results which could not be properly scientifically interpreted, and was abandoned as a tool to estimate timing of the resumption of oestrus. No other method for the estimation of return to oestrus was used during the 2007 breeding season.

Figure 4-1 shows the progesterone curves post-calving in 2008 for each animal in each replicate group. It gives an impression of the variation in “baseline” progesterone measures at the first sample point.

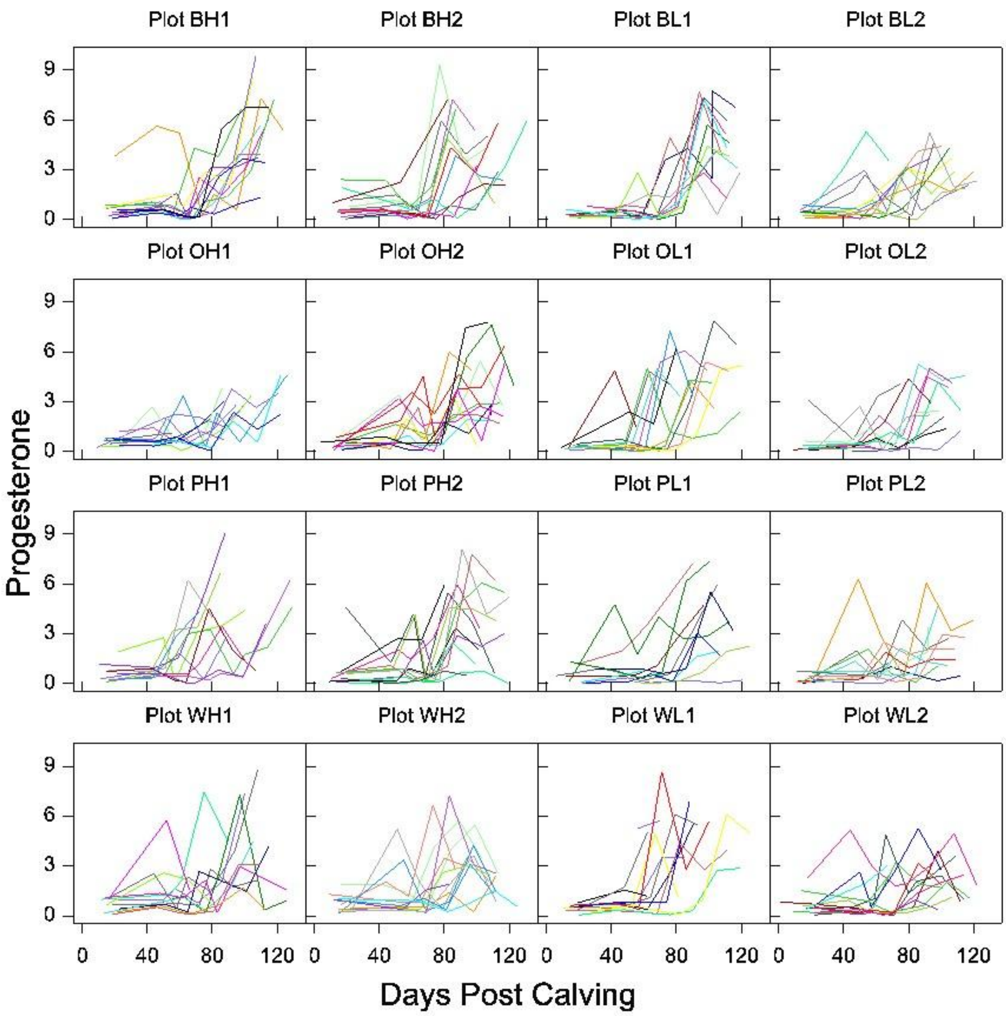


Figure 4-1: Trellis plots of progesterone concentrations (ng/ml - y-axis) vs. days-post-calving, by replicate group.

Table 4-1 shows the levels of significance for the LMM used to analyse days to first ovulation. Neither Genotype nor nutrition had a significant effect on the number of days to first ovulation as estimated by the methodology described in this chapter. This estimate of PPAI was affected by the age of the animal ($P < 0.001$) with the first cohort of animals taking 12.2 days longer to ovulate. Calving date affected PPAI ($P < 0.001$) whereby the later the date of calving, the shorter the length of the PPAI. For delay of one day in calving the PPAI reduced by 0.5209 (SE ± 0.09265) days.

Table 4-1: Analysis of days to first ovulation.

progesterone change 2008	**days-post-calving**
location	0.191
cohort	**<0.001**
line	0.137
FatvsLean	0.306
HiNFIvsLoNFI	0.885
calving date	**<0.001**
3rd trimester P8	0.600
nutrition	0.113
line.nutrition	0.213
FatvsLean.nutrition	0.354
HiNFIvsLoNFI.nutrition	0.721

4.6 Discussion

The accuracy of physical methods such as Estrus Alerts to detect return to oestrus is variable in the best circumstances (Diskin and Sreenan, 2000). Results of the current experiment showed that when Estrus Alerts were used in 2007, the combination of the inclement weather conditions, the lack of observational records, the infrequent mustering and the difficulty in the interpretation of the rubbings led to the conclusion that it was impossible to estimate PPAI in the cow using this method. For this reason another method was used in 2008, with limited success. Estrus alerts are far more useful in a dairy herd where animals are handled and checked every day.

In the experiment the restriction was that samples were taken from the cows no more frequently than every two weeks during the post calving period as described in Section 2.9.3. This meant a limitation to an interpretation of minimum fortnightly progesterone assay results. The assumption was made that a measurement of serum progesterone significantly increased (>2ng/ml) above the baseline level would be indicative of a previous ovulation.

Figure 4-1 shows there was considerable variation between cows in the concentration of progesterone in the first measure, taken approximately two weeks post-calving when any resumption of oestrus was unlikely to have occurred. These "baseline" concentrations were sometimes above a level which other studies have reported to be indicative of a prior ovulation (Echternkamp and Hansel, 1973; Garmo *et al.*, 2008; Garmo *et al.*, 2009). Whether these high values resulted from the assay methodology or just a large variation among animals in the herd, they made it impossible to have a "cut-off" concentration indicative of an ovulation, as many studies do. However, most cows did show a subsequent increase in progesterone concentration, so it was decided that when an animal had an increase for the first time of >2ng/ml from its baseline measure, made within 2 weeks of calving, it would be deemed to have ovulated.

This definition caused two problems. Firstly it excluded all animals which had no significant increase in progesterone. Out of 200 experimental animals, only 159 cows were identified as having had an ovulation in the period from calving until the end of joining. This result is clearly wrong as subsequent pregnancy rates, which were above 90% for all Genotypes, indicate that the number of animals that not only ovulated but also conceived was far greater than that identified by the progesterone assay method. The probable reason for this is that because samples were at best taken fortnightly, there would have been a percentage of animals which, despite having ovulated previously when the sample was taken, would have been at the very start or the very end of an oestrous cycle, when progesterone concentrations would have been low, i.e. not significantly increased from the baseline.

It has been suggested that the optimum frequency for sampling progesterone in beef cattle to detect oestrus activity is twice a week (Bolaños *et al.*, 1997; Kyle *et al.*, 1998). With more manpower and the financial freedom to analyse more samples it may have been possible to sample the animals more frequently, but the key premise in the whole experiment was to gain an understanding of the efficiency of animals of differing genotypes on an extensive grazing system. Mustering events are stressful and likely to disrupt the normal intakes of the animals. Walks to and from yards were up to 1.5km so the decision was made early on in the experiment to have a maximum of one mustering event per fortnight to minimise the impact on group intake measures.

The second problem was that there was no other indicator of oestrus that supported the identification of cows as having returned to oestrus. It was concluded that although serum progesterone concentrations post-calving have been used to predict the length of the PPAI, fortnightly measures are inadequate for this purpose. Moreover, in the absence of any record of oestrous behaviour or physical indicator such as an Estrus Alert, it was not possible to get any accurate estimate of an individual PPAI.

The exercise was useful in that it highlighted the significant effect of age and calving date. Younger cattle took longer to return to oestrus perhaps owing to the greater energy deficit post-calving as well as their lower body fat reserves compared to those of the older cows. The young cattle were also subject to the energy demands of growth which the older cattle were not, and given the influence of energy balance on PPAI the result is unsurprising. It was notable that in cows whose calving date was closer to the end of the calving period, PPAI was shorter. This was probably a function of energy supply. As the season progressed in 2008, energy became more available because of the increase in pasture growth. Warmer temperatures contributed to this as the season progressed and the last calves were born well into spring, at the peak of the growing season. This effect of calving date on indicators of energy supply is also seen in the analysis of the biochemical parameters, discussed further in Chapter 6.

In unpublished work (Savage *et al.* 2010, *Oestrus detection in beef cows*, In preparation) it was shown that when several methods of oestrus detection in beef cows were used simultaneously it was still very difficult accurately to predict resumption of oestrus. Different behavioural and physical methods were used including observation of standing, licking, mounting and nuzzling behaviour, as well as measures of vaginal temperature, serum progesterone, ovarian activity via ultrasound, and Kamar® heat detectors. The methods did not correlate well and when compared to each other they reduced the accuracy of oestrous prediction of the others. It was concluded that even using only one method, detection of the resumption of oestrus in a beef breeding herd is difficult and the serum progesterone method does not yield accurate results if measured only once a fortnight.

It was noted that the effect of cohort, or the age of the cows, was not significant in the analysis of DTC (see Section 5.5.1), highlighting the reduced power in the experimental design to detect significant differences in DTC. It also reflects that DTC is influenced by not only the PPAI but also gestation length and the conception rate at first ovulation.

Calving date was a factor that reduced the PPAI. The results in the current experiment showed that as time went on and cows calved later in the season, their PPAI was reduced. This result is consistent with other research (Yavas and Walton, 2000) and is probably a function of the increased availability of feed later in the year and the lessening of the energy deficit suffered by the animals during lactation. Animals also calved in better body condition the later they calved (for every day post calving P8 increased by 1mm ± 0.02, $P = 0.003$), which would have provided them with more energy reserves to overcome the energy demands of lactation and returning to oestrus. Lactation prolonged the PPAI which is a direct consequence of the increased energy demand during post-calving.

The PPAI is governed largely by the degree of energy deficit post-calving but the actual length of the PPAI is difficult to measure accurately in an extensively grazing beef herd. Although the experiment provided a useful analysis of the effect of energy deficit on PPAI, it did not yield significantly accurate measures of the PPAI among the treatments to use in a comparison of the main effects of Genotype and nutrition.

CHAPTER 5. PRODUCTION MEASURES AND HERD EFFICIENCY IN A BEEF PRODUCTION SYSTEM USING COWS SELECTED FOR A DIVERGENCE IN FATNESS OR FEED EFFICIENCY

5.1 Introduction

The income derived from a beef cow/calf enterprise is from the sale of animals as meat from young cattle or culled cows (Lucy, 2004), or as live cows to be used as breeders on other farms. The number of kilograms yielded from a production system is determined by the inputs: in particular the type, cost and amount of feed consumed by the animals; the genetics of the cattle and their predisposition towards such things as leanness or fatness, rapid growth, muscularity, marbling and high or low birth weights; their breed; twinning rates; fertility of breeding cattle, and their return to service after calving. Above all, the most influential factor is the nutritional management of reproducing cows and their calves (Zulu *et al.*, 2002; Hess *et al.*, 2005). Because of the high cost of feed, it is not surprising that an ability to select cattle that eat less food for the same level of production is attractive to producers. The identification of NFI as a heritable trait has been crucial in achieving this (Crews, 2005). Similarly, animals that yield more meat per carcass are more profitable, so the ability to select for leanness, which is associated with higher lean meat yields, is of economic benefit to beef producers. This benefit, however, has to be weighed against the increased maintenance requirements of lean animals compared to fatter animals (DiCostanzo *et al.*, 1990; DiCostanzo *et al.*, 1991; Egan *et al.*, 2001; Walmsley and Parnell, 2009), especially as feed input is such a large proportion of the total input in a cow/calf enterprise.

The focus of this chapter is the consideration of production and efficiency parameters important to a beef cow/calf producing, pasture-based enterprise in the South West of WA, and how these parameters are affected by selection for fatness or feed efficiency. In this region, breeding beef cows are raised on extensive grazing systems, and supplementary feed is added at certain times of the year to account for the lack of FOO in paddocks during the dry season. The experiment included data from two parities, 2007 and 2008, of cows on an extensive grazing system at Vasse Research Centre, Busselton, WA, and was designed to reflect the conditions that may be experienced by a cow/calf producer in this area. A nutritional restriction treatment was included to mimic conditions where the supply of energy is limited, for instance in times of drought. The experiment considered production parameters and efficiency measures and the effect on those parameters of particular genetic selection and level of nutrition.

5.1.1 Production parameters

Measuring reproductive traits and producing genetic breeding estimates in beef cattle is difficult, in part because reproductive potential is often constrained and heavily influenced by the management system. Reproductive data is complex, and is gleaned from many events occurring throughout the breeding season. DTC is defined as the interval in days between the first joining date each year for a cow under paddock joining conditions and subsequent calving (Meyer *et al.*, 1990; Johnston and Bunter, 1996). DTC is used in the beef-producing industry as a measure of female fertility, and is included in the national genetic evaluation scheme Breedplan because it is both heritable and cost effective to measure (Johnston and Bunter, 1996). It was included in Breedplan in 1993 (Schneeberger *et al.*, 1991) as a trait with an attached EBV using parameters described by Meyer *et al.* (1990). Several factors influence DTC including herd, year and month of joining, service sire, previous joining season and age at joining (Johnston and Bunter, 1996). DTC as a trait is useful to producers because it varies according to many aspects of the production system, is easy to measure, and is heritable. In this experiment it was used among other measures to assess MP in the experimental cattle.

Birth weight of calves is an important parameter in an extensive grazing beef enterprise, mainly because of its association with dystocia in beef cows (Hickson *et al.*, 2006). Dystocia is defined by Rice (1994) as ‘a birth that reduces calf viability, causes maternal injury, or requires assistance’. In an extensive grazing system any need for assistance at calving is especially undesirable: the herd is generally dispersed over a wide area and calving time is already a labour intensive period in the breeding season. Dystocia is responsible for the death of both calves and occasionally cows when undetected, and is a source of major economic loss (Meijering, 1984). It is well recognised that calves that experienced dystocia were heavier than calves that experienced a normal birth (Arthur *et al.*, 2000), and there is a high genetic correlation ($r = 0.98$) between birth weight and dystocia in beef cattle (Meijering, 1984).

The conclusions drawn in the literature describing the effect of the prepartum level of nutrition of the dam on birth weights of calves are contradictory. It has on the one hand been shown that cows fed on a higher plane of nutrition during the first trimester of pregnancy gave birth to heavier calves than those on a low level of nutrition pre partum (Bellows and Short, 1978; Wiltbank and Remmenga, 1982; Pleasants and Barton, 1992; Spitzer *et al.*, 1995; Cafe *et al.*, 2006), while other reports show prepartum levels of nutrition making no difference to calf birth weights (Bellows *et al.*, 1982; Lake *et al.*, 2006). Micke *et al.* (2010) report that low-nutrition during pregnancy results in calf birth weight decreases ($P < 0.05$) and decreased crown-rump length at day 39 ($P < 0.001$). Low birth weights is still a desirable trait in the female herd because of the potential to reduce the incidence of assisted calving (Bellows and Short, 1978).

The growth rate of calves, or average daily gain in kilograms (ADG) until weaning, is also associated with the level of nutrition of the dam. Calves grow faster when dam nutrition is better (Cafe *et al.*, 2006). These researchers concluded that the nutritional level during lactation had a greater effect on ADG than nutritional level during pregnancy, but others (Micke *et al.*, 2010) postulate a significant link between maternal nutrition during pregnancy and neonatal growth rates. Either way, increased nutrition during pregnancy and lactation contributes to an increased ADG of calves. The better quality of the pasture on the high-nutrition treatment would have also contributed to the difference in the growth rates. The difference in ADG between high- and low-nutritional treatments extrapolates to weaning weight (Martin *et al.*, 2007), but not necessarily finishing weight in beef calves (Drouillard and Kuhl, 1999; Stalker *et al.*, 2006). This can be explained by the 'compensatory growth' hypothesis. First described by Bohman (1955), compensatory growth is the term that describes the accelerated and/or more efficient growth that commonly follows a period of growth restriction. Usually it is considered in the context of recovery from nutritional deprivation and would apply particularly when nutrition during suckling has been restricted. Calves that grow faster are heavier at weaning when corrected for birth weight. Growth rate of calves is an important production parameter because it affects weaning weight. It reflects the nutritional input of milk production from the dam.

5.1.2 Efficiency parameters

Profits in a beef enterprise are governed by not only the return realised from the outputs such as kilograms of beef weaned but also by the cost of the inputs. Providing feed to animals is one of these major input costs and includes the cost of land, pasture improvement, fertiliser, irrigation, supplementary feed, operating and capital costs of plant, machinery, and labour used in feeding (Archer *et al.*, 1999). It has been estimated that the provision of feed accounts for between 66% and 77% of the total cost of weight gain in a cow/calf producing enterprise (Anderson *et al.*, 2005). Increasing the output of beef per unit of feed is of great economic benefit to the enterprise.

Different indices of efficiency are used to compare animals, or groups of animals, within a production system and generally compare feed input with various production outputs over a specific period of time in the production system (Archer *et al.*, 1999). Finding the appropriate index to describe the efficiency of the production system is difficult. One must include energy (feed) input of both the breeding and the slaughter generations and compare this to the production outputs such as kilograms of beef weaned. The calculations should also take into account calf mortality, and reproductive ability such as conception. Archer *et al.* (1999) suggest that it is simplistic to use an index during a restricted phase of the production cycle as representative of an individual's or a group's efficiency in the context of the entire production system but for the purposes of the current experiment this is what occurred. Because the NFI trait distinguishes between animals that eat more or less than the average for the same gain, it was decided to test the efficiency of animals selected for this trait on a grazing system. The other trait tested was selection for a divergence in fatness.

The associations between maintenance requirements of livestock and fatness have been reported (Cleveland *et al.*, 1983; DiCostanzo *et al.*, 1990) with fatter animals having lower maintenance requirements than lean animals at similar liveweights (NRC, 1996), although synthesis of protein is a more energy efficient process than lipogenesis (McDonald *et al.*, 1988; DiCostanzo *et al.*, 1990). Using average body composition values in Angus cattle, DiCostanzo *et al.* (1990) estimated the energy required for fat and protein maintenance, and calculated that of the total energy requirement for maintenance, 88.6% was used to maintain protein and only 11.4% was used to maintain fat. More energy-efficient cows have been shown to have lower maintenance requirements (DiCostanzo *et al.*, 1991) and preferentially to store and retrieve body energy in the form of fat rather than protein (DiCostanzo *et al.*, 1991; Basarab *et al.*, 2007). These results suggest maintenance requirements for leaner animals are likely to be higher than for fatter animals and lead to fat animals being more efficient on pasture grazing systems. The compromise is the price penalty paid by producers for fat rather than lean meat when animals are sold to slaughter (Egan *et al.*, 2001). An understanding of the effect of selection for fatness on efficiency was considered important, particularly in helping beef producers to make management decisions.

Efficiency parameters that are considered in this chapter include: DM intake (assessed as DM disappearance and representative of the total kg of dry matter consumed per cow/calf unit/ per day during the pasture grazing season), MJ ME consumed (assessed as MJ ME disappearance and representative of energy consumed per cow/calf unit/day during pasture grazing season), total kg weaned (total kg weaned per treatment) and MJ ME consumed per kg beef weaned.

5.2 Aims

The aims of this experiment were to:

1. measure intake of beef cows grazing green pasture;
2. measure production parameters and use intake measures to produce efficiency indices in female beef cattle of differing Genotypes;
3. determine whether different Genotypes of cattle differed in terms of MP and whether level of nutrition influenced MP differently depending on animals' Genotype;
4. identify if the benefits of selecting cattle for increased feed efficiency, or leanness, are notable and persist in an energy-restricted environment.

5.3 Hypotheses

It was hypothesised that:

1. measures of pasture intake would be highly variable;
2. there would be no difference between Genotypes in DTC, birth weights, growth rates and weaning weights of calves, but nutrition would impact on all of these;
3. Fat and low-NFI animals would have lower DM intake, reflected in measures of DM disappearance from pastures, than Lean or high-NFI animals. This would translate to fewer MJ ME being consumed on average by Fat and low-NFI cows;
4. nutritional treatment would affect DM disappearance, MJ ME consumed and MJ ME consumed / kg beef weaned;
5. on high-nutrition Fat and low-NFI animals would consume fewer MJ ME/ kg beef weaned than Lean or high-NFI animals;
6. on low-nutrition Lean and low-NFI animals would consume more MJ ME/ kg beef weaned than Fat or high-NFI animals;
7. animals selected for decreased fatness, or superior feed efficiency, would maintain MP in good, but not in poor, nutritional environments, and the benefits of selecting for these animals would be lost when energy intake was restricted.

5.4 Materials and methods

5.4.1 Animals

The animals used in this experiment are as described in Section 2.1. Table 2-1 outlines the experimental design and allocation of animals to the experiment. In 2007 only the 1st cohort of animals had calved and therefore the data in this year was sourced from the 1st cohort only, whilst in 2008 both cohorts were included in the data set.

5.4.2 Data collection

Liveweight and ultrasound measures of fatness

All cattle were weighed approximately once a fortnight. US was used to measure subcutaneous fat deposits at the Position 8 (P8) site. All experimental animals had US measurements taken during the breeding season. In 2007 there was a single scan in May, about one month before the start of calving; thereafter calving began and there was a two week period in the middle of the calving period when all animals were scanned. The animals were scanned again at the end of the joining period. Measurements were taken more frequently in 2008: the first pre-calving scan was conducted about one month before calving began and thereafter scans were conducted monthly from May to December. The procedure was carried out with the animal standing in a crush with the operator standing on the left hand side (see Figure 2-4). The ultrasound machine was a Pie Medical Scanner 200 RTUS machine equipped with a 17.2-cm, 3.5-MHz linear transducer probe which was used to obtain the measurements.

Birth weight recording

The birth weight of each calf was recorded a maximum of 16 hours after birth. A leather sling and hanging clock-face scales (QWM / Accuweigh Pty Ltd**,** Geebung, Queensland, Australia) mounted on a frame on the tray of a farm utility vehicle was used to restrain and weigh the calves (see Figure 2-5). The calves were weighed in the paddock in which they were born. Each calf had its sex and weight recorded, and was ear-tagged with a sequentially numbered ear tag. At the time of weighing male calves were castrated using Elastrator Rings© (Nasco International, Fort Atkinson, Wisconsin, USA) placed at the base of the scrotum.

5.4.3 Estimation of group feed intake on growing pasture

Estimates of group feed intake were made during the 2008 green pasture grazing seasons between the months of May and November. This was done using measures of pasture disappearance. FOO was measured before and after a replicate group had grazed a particular paddock. This then provided an estimate of how much pasture disappeared while the replicate group was grazing the paddock. The measure was recorded as DM disappearance (DM disappearance) which was used as representative of DM intake in the analysis in the experiment.

No estimates of group intake were made during 2007 green pasture grazing season.

Measurement of FOO

Two methods were used to estimate FOO during the green pasture grazing season. The first used a visual assessment of the pasture in association with calibration cuts and the second used a bike-mounted Ellinbank Automatic Pasture Reader (Victoria Department of Primary Industries) and the associated software Reader Version 2.0 – for Pasture Reader firmware v1.7 (Victoria Department of Primary Industries). This machine takes several laser pasture height measurements per second and was driven across the sample area using an all-terrain four-wheel motorbike (see Figure 5-1) in order to get an accurate average height over the whole sample area. This height then correlated with a particular kg DM/hectare (MLA, 2004). When results from the Ellinbank Automatic Pasture Reader were compared to pasture yielded from several mower cuts and converted to a measurement of kg DM/ha, there was an R^2 of 0.94 (Naroaka, 2006). This result suggested that DM/ha can be accurately predicted with the Ellinbank Automatic Pasture Reader.

The visual assessments were done by the same trained team members and provided an estimate of pasture height that corresponded to a kg DM/hectare measure. Calibration cuts were made weekly on all growing pastures and fortnightly on dry pasture in order to estimate dry matter from visual estimates. Cuts were made with a strip mower and samples were stored in plastic bags and refrigerated no more than one hour post collection. More than ten calibration cuts were done each time the measurements were taken in order to cover the full range of dry matter levels. Good correlations between actual and visual estimates of dry matter were achieved (r^2 = 0.61 to 0.99).

Figure 5-1: Bike-mounted Ellinbank automatic pasture reader (Victoria Department of Primary Industries)

Estimation of pasture growth rates

Estimation of the growth rate of pasture is historically difficult. It can be done by using pasture growth cages where an area of pasture is fenced or caged off from the grazing herd and measures of pasture height taken at regular intervals. The difficulty with this method is that grazed pasture grows at a different rate to un-grazed pasture. Another method is to use Pastures From Space (CSIRO, Western Australia, 2006). This programme provides estimates of pasture production during the growing season by means of remote sensing. Satellite data is used to accurately and quantitatively estimate pasture biomass or FOO or, in conjunction with climate and soil data, is used to produce PGR estimates. The difficulty is that the programme is not sensitive enough to distinguish between small paddocks, and the design of this experiment dictated that paddocks were either approximately 6.3 or 3.6 hectares, too small for the programme to work.

With the inherent inaccuracy of measuring pasture growth rates on a small scale and at different grazing intensities, it was decided to disregard pasture growth in the estimation of DM intake in the cattle. An assumption was made that the pasture growth rates on the different nutritional treatments, although different between treatments, would have been the same across all replicate groups within a treatment. This meant that differences in pasture disappearance between Genotypes would be the same as differences in DM intake. Thus rather than comparing Genotypes using values of DM intake which used an estimate of pasture growth that is inaccurate, Genotypes were compared using pasture disappearance. No conclusions were drawn about the actual amount of pasture eaten by the different genotypes; rather differences in the disappearance of DM for each measured period were recorded and analysed to determine if the different Genotypes were eating different amounts. No conclusions were made about differences in DM intake between nutritional treatments, but pasture disappearance was analysed to see if the nutritional treatments appeared to be affecting this parameter.

Estimation of DM disappearance

The following formula describes how an estimation of DM disappearance was arrived at for a single grazing period and replicate group.

$$\text{DM disappearance (kgDM/hd/day)} = \frac{\text{DM disappearance reading x paddock area}}{\text{number of animals x number of days}}$$

A weighted DM disappearance average was calculated for each replicate group over all the grazing periods from 21 May to 23 December, 2008. The methodology yielded a total of 16 measurements across the experiment, one for each replicate group (4 Genotypes x 2 nutritional treatments x 2 replicate Groups).

Determination of pasture quality

Pasture quality was assessed from regular pasture cuts over the course of the green pasture grazing period, from May to December in 2008. Samples were sent to the laboratory at the Department of Agriculture and Food, Bunbury, Western Australia for analysis (Aufrere and Michalet-Doreau, 1988; AFIA, 2005). Values for DM (%), crude protein (%), dry matter digestibility (%), ash (%), organic matter (%), organic matter digestibility (%), dry organic matter digestibility (%) and metabolisable energy (ME/kg) were provided in the output of all pasture quality analyses.

Since cuts for pasture quality were not necessarily made continuously from the same paddocks, the paddocks within each farm have been grouped together according to their nutritional treatment and pasture types. Quality measurements were examined within each group (see Figure 5-2). There were four paddock groupings within farm A (H1, H2, H3 H4) and another four within farm B (M1, M2, M3, M4).

Estimation of MJ ME eaten

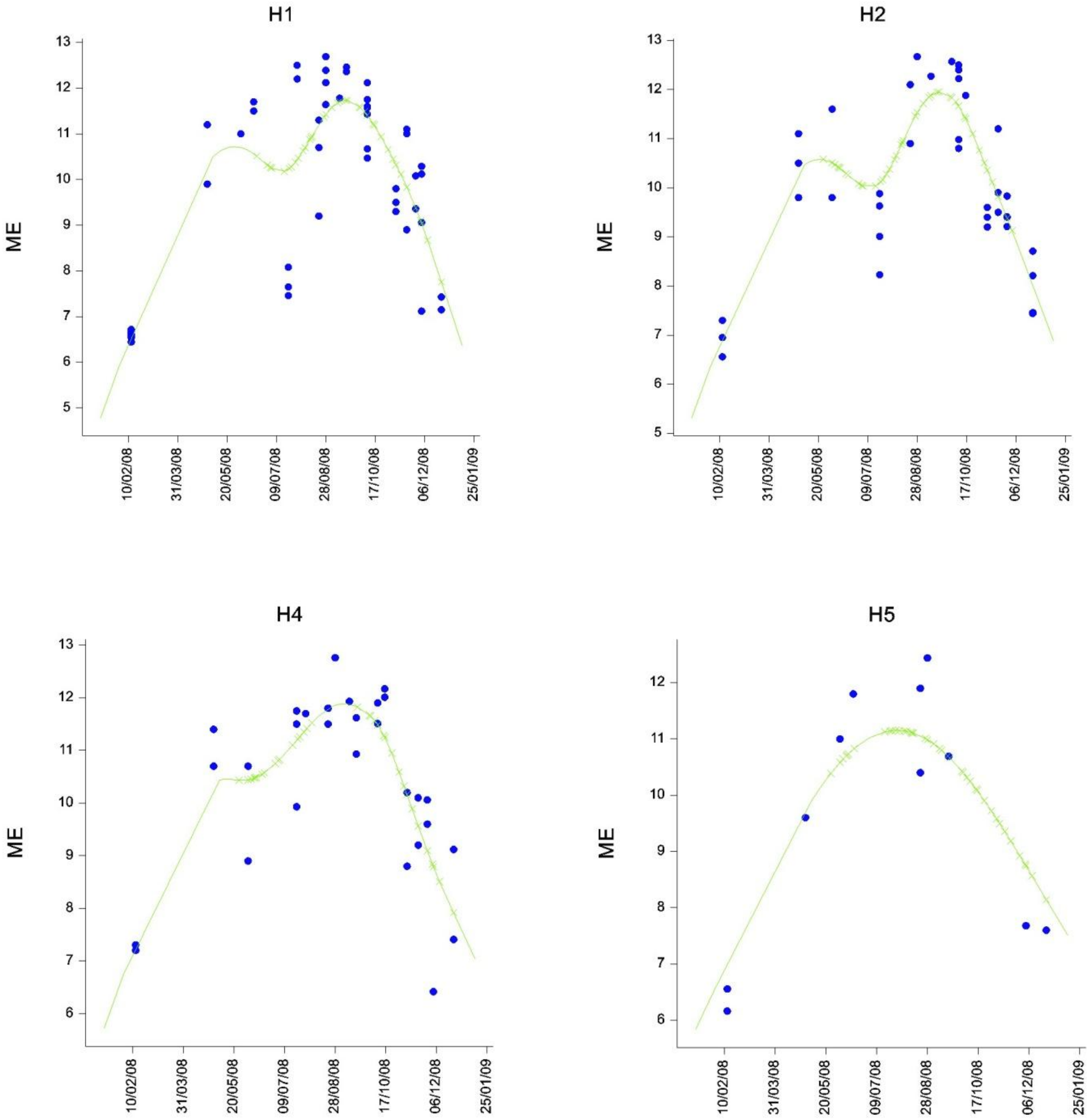
The number of MJ ME consumed by the replicate groups was estimated by converting measures of DM disappearance across the grazing period into MJ ME disappearance. This was done on the basis of the pasture quality results from the samples taken at various times of year and within each paddock grouping. Quality measures were not necessarily recorded for every date and paddock on which measures of DM disappearance were derived, so in order to match a measure of DM disappearance with a quality (ME) measure, trend lines were fitted to the ME data for each paddock grouping (see Figure 5-2). Splines were used for the trend lines. Splines are functions, constructed from segments of cubic polynomials. The segments are constrained to be smooth where they join, at values of the function variable known as knots. The result is a smooth flexible curve which can model a relationship which may not follow a simple curve (Ferguson, 1964). Using the splines, MJ ME estimates were produced for each date upon which a DM disappearance estimate was recorded.

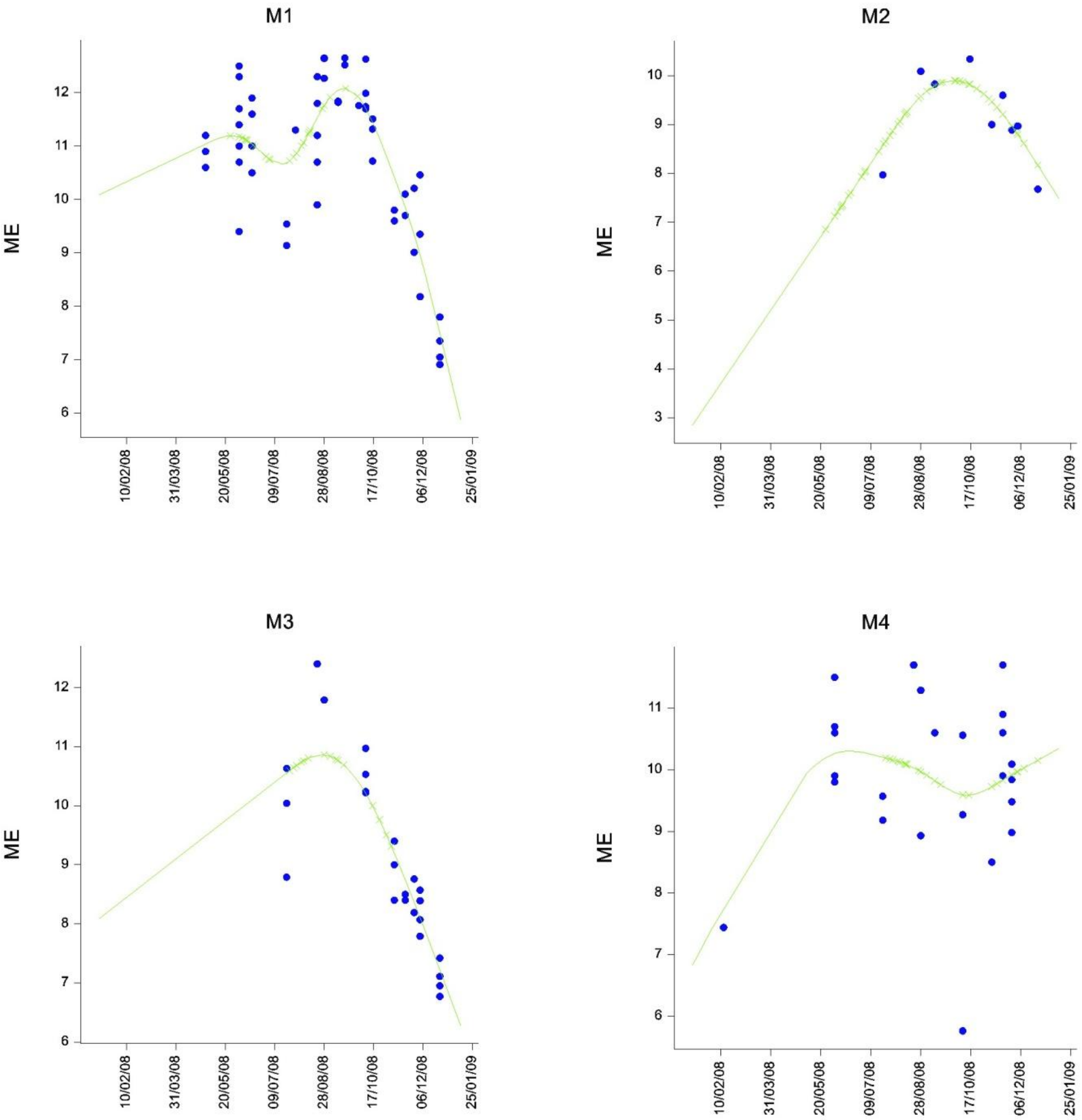
The conversion from DM disappearance to MJ ME disappearance for a single grazing period and replicate group was:

MJ ME disappearance /head/day = DM disappearance x predicted MJ ME measure.

A weighted MJ ME average was then calculated for each replicate group over all the grazing periods from 21 May to 23 December, 2008.

Figure 5-2: Splines used to predict MJ ME on similar groups of paddocks (Farm A – replicate groups H1,H2,H3,H4 and Farm B – replicate groups M1,M2,M3 and M4). Blue dots = actual MJ ME measure and green x = predicted values for dates corresponding to DM measures. Y-axis ME (MJ ME).




M1
M2
M3
M4
ME
10/02/08
31/03/08
20/05/08
09/07/08
28/08/08
17/10/08
06/12/08
25/01/09

5.4.4 Statistical Analysis

For a general description of statistical analyses and an explanation of fixed and random terms in the models described below see Section 2.10.

DTC

A LMM with the following fixed and random effects was used to identify significant effects of Genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition and their interactions on DTC.

Fixed model:

constant + *location* + *cohort (2008 only)*+ *multiple birth* + *calf sex* + *line* + *FatvsLean* + *HiNFIvsLoNFI* + *dam birth date* + *nutrition* + *line.nutrition* + *FatvsLean.nutrition* + *HiNFIvsLoNFI.nutrition*

Random model:

replicate + *dam ident*

The effects of Genotype and nutrition were corrected for location, cohort, multiple birth (2008 only) and calf sex. In addition, the main effect of nutrition and interactions between Genotype and nutrition were corrected for the age of the dam.

Calf parameters: birth weight, growth rate and weaning weight

A LMM with the following fixed and random effects was used to identify significant effects of genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition and their interactions on the birth weights, growth rates and weaning weights of calves. Average growth rate per calf (kg/day) was calculated as the slope of a regression between weight and date from birth until weaning.

Fixed model :

constant + location + cohort (2008 analysis only) + calving date + multiple birth + calf sex + line + height + FatvsLean + HiNFIvsLoNFI + dam birth date + 3rd trimester P8+ nutrition +line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition

Random model :

replicate + dam ident + calf ident

All effects of Genotype and nutrition were corrected for location, cohort (2008 analysis only), multiple birth (2008 analysis only), calf sex, height, and calving date. In addition the main effect of nutrition and interactions between nutrition and Genotype were corrected for age of dam and 3rd trimester P8. Calf parameters were corrected for 3rd trimester P8 of the dam so that any Genotype effect on parameters was not due to a difference in fatness of the dam.

Efficiency parameters - DM disappearance, MJ ME disappearance and MJ ME per kg weaned.

Results for each of these parameters were obtained for each replicate group (16 in total). These results were then analysed using a regression analysis using the following model:

Fixed model:

constant + %lact + nutrition + line + HiNFIvsLoNFI + FatvsLean + line.nutrition + HiNFIvsLoNFI.nutrition + FatvsLean.nutrition

Random model:

replicate group

Main effects of Genotype and nutrition, and interactions were corrected for % lactation (the percentage of cows lactating within each replicate group).

The model structure and level of significance (P-values) for all statistical models used in this thesis are shown in Appendix 9.6.

5.5 Results

The effect of Genotype and nutrition on production parameters is described below. Figures show treatment means across both years of the experiment. Significant differences between either Genotype or nutritional treatment are highlighted only within year, not between years. On the bar graphs, where results for all four groups (two lines, two Genotypes per line) are illustrated, the valid comparisons are made between Genotypes only within lines (i.e. Fat vs. Lean; high-NFI vs. low-NFI). Truncated y-axes are used in this section to give a better impression of variation.

The associations between covariate measures and measured traits are presented in tables. Each trait is presented with a covariate effect and P-value to indicate the level of significance of the association between the covariate and the measured trait. Effects are presented such that an increase of 1 unit of covariate measure results in change in the measured trait. The degree of change is represented as a figure in a column titled "Effect".

Error bars on bar charts represent Standard Errors. These are included to give an impression of variation around each mean. They are not Least Significant Differences and thus comparison of error bars does not always indicate significant ($P < 0.05$) differences between means.

5.5.1 Days-to-calving

DTC was not affected by Genotype in 2007 or 2008 but was significantly ($P < 0.05$) affected by nutrition in 2008 (Figure 5-3). In this year, dams on the high-nutrition treatment had an average DTC of 329 ± 2.785, while low-nutrition animals had an average DTC of 335.1 ± 2.887. Age of dam (cohort) did not affect DTC, but in 2007 dam birth date did significantly affect DTC ($P = 0.013$), see Table 5-1.

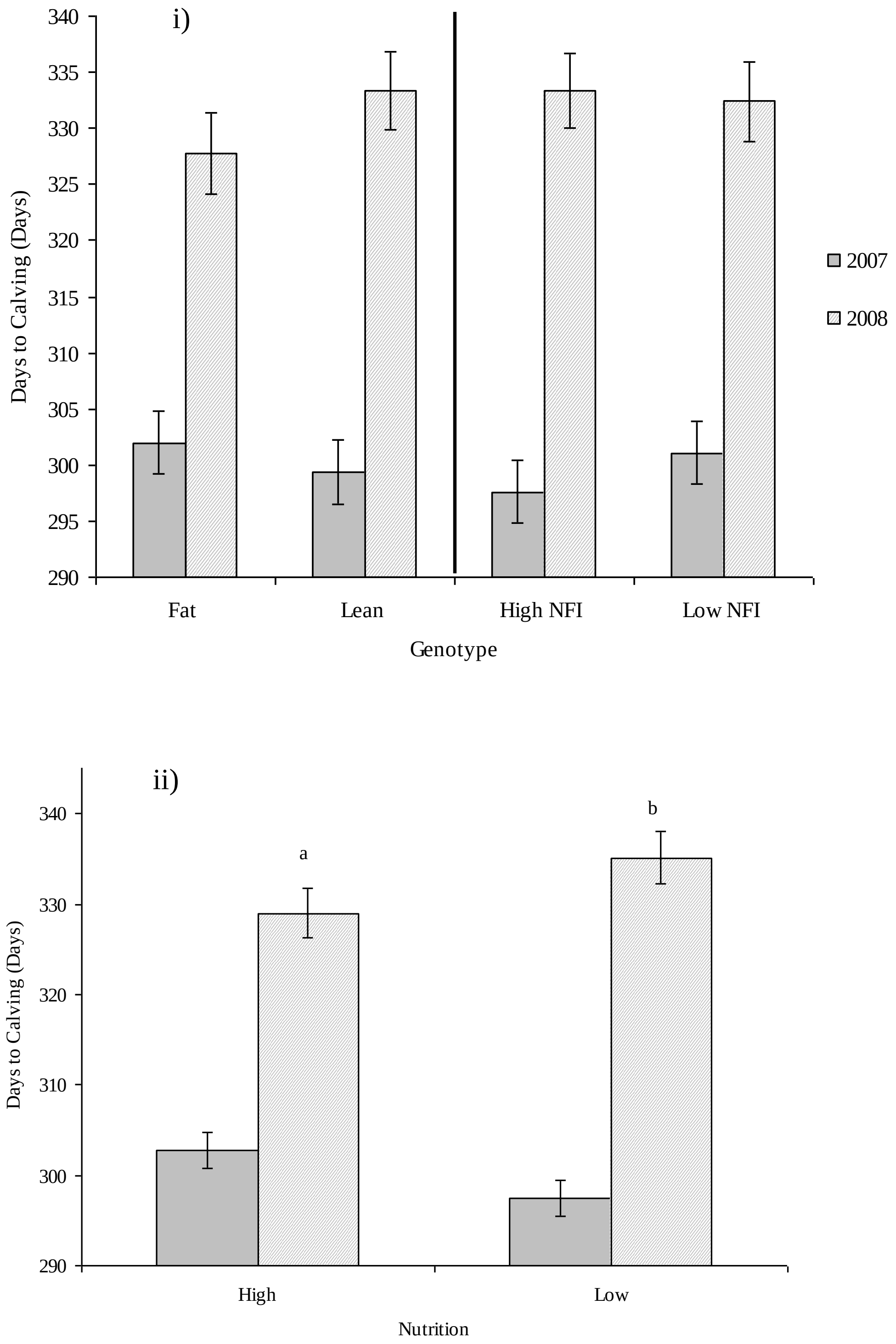


Figure 5-3: Main effects of (i) Genotype (corrected for calf sex and multiple birth) and (ii) nutrition (corrected for dam birth date, calf sex and multiple births) on DTC (days) in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent standard errors.

Table 5-1: Effect and level of significance (P-value) of covariates (calving date, height, dam-birth-date and 3rd trimester P8) on production parameters in 2007 and 2008.

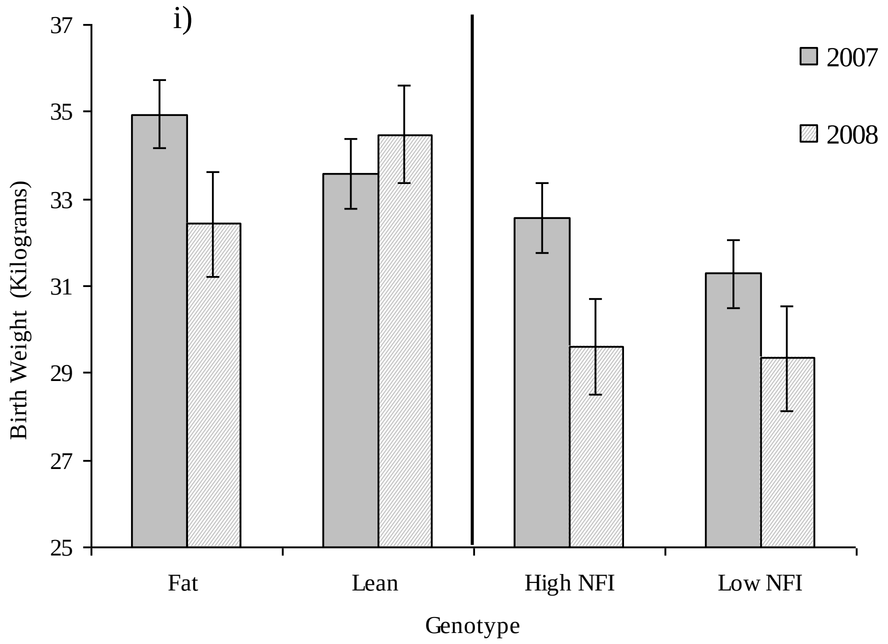
	2007							
	calving date		height Aug 07		dam birth date		P8 May 07	
	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value
birth weight	0.18	**<0.001**	0.10	0.770	-0.03	0.190	-0.05	0.819
calf growth rate	0.0007	0.213	0.0041	**0.055**	-0.0005	0.220	-0.0014	0.649
weaning weight	-0.89	**<0.001**	1.01	**0.038**	-0.07	0.431	-0.32	0.641
DTC	N/A	N/A	N/A	N/A	0.1393	**0.013**	N/A	N/A
	2008							
	calving date		height Jan 08		dam birth date		P8 April 08	
	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value
birth weight	0.09	**<0.001**	0.06	0.171	0.01	0.707	0.01	0.542
calf growth rate	-0.0003	0.676	-0.0015	0.352	-9E-05	0.917	-0.0019	0.730
weaning weight	-1.04	**<0.001**	-0.34	0.287	-0.04	0.741	-0.38	0.639
DTC	N/A	N/A	N/A	N/A	0.04229	0.452	N/A	N/A
calf P8 at weaning	-0.02	**0.001**	-0.05	0.113	-0.08	0.352	-0.02	0.393

Significance values (P-values) and LMM model structure for the analysis of DTC are shown in Table 9-5 and Table 9-6.

5.5.2 Birth weight

The birth weight of calves was not affected by Genotype or nutrition in 2007 or 2008 (Figure 5-4). Birth weight was affected in both years by calving date such that calves born later were heavier ($P < 0.001$, see Table 5-1). Male calves were heavier ($P < 0.05$) than female calves (Figure 5-5) in both years, and twins, which only occurred in 2008, were lighter ($P < 0.001$) than single births (results not shown). Height of dam and 3rd Trimester P8 had no effect on birth weight in either year (Table 5-1).

Significance values (P-values) and LMM model structure used for the analysis of birth weight are shown in Table 9-5 and Table 9-6.



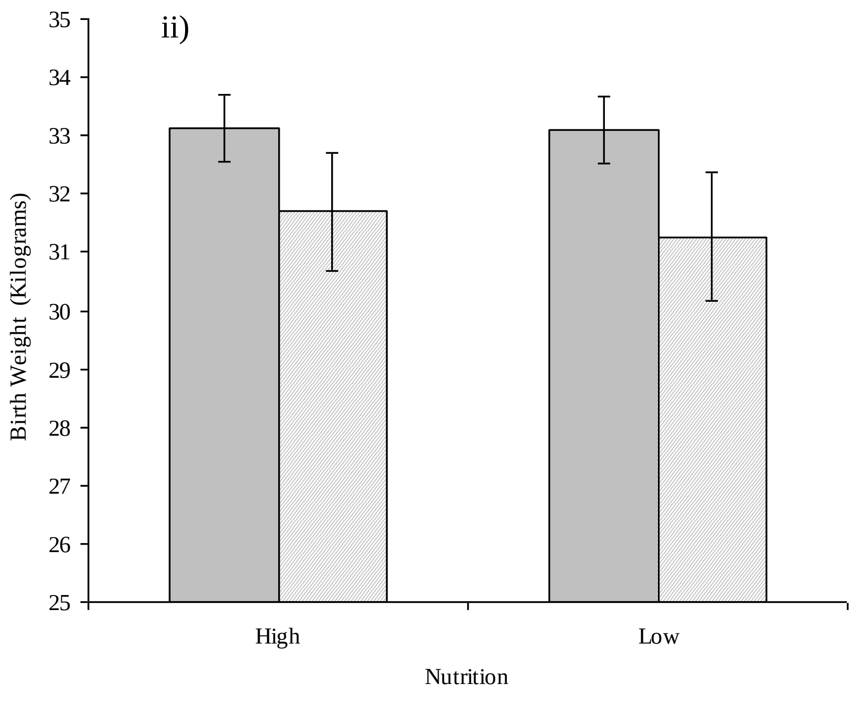


Figure 5-4: Main effects of Genotype (i) and nutrition (ii) on birth weight (kg) in 2007 and 2008. Error bars represent standard errors.

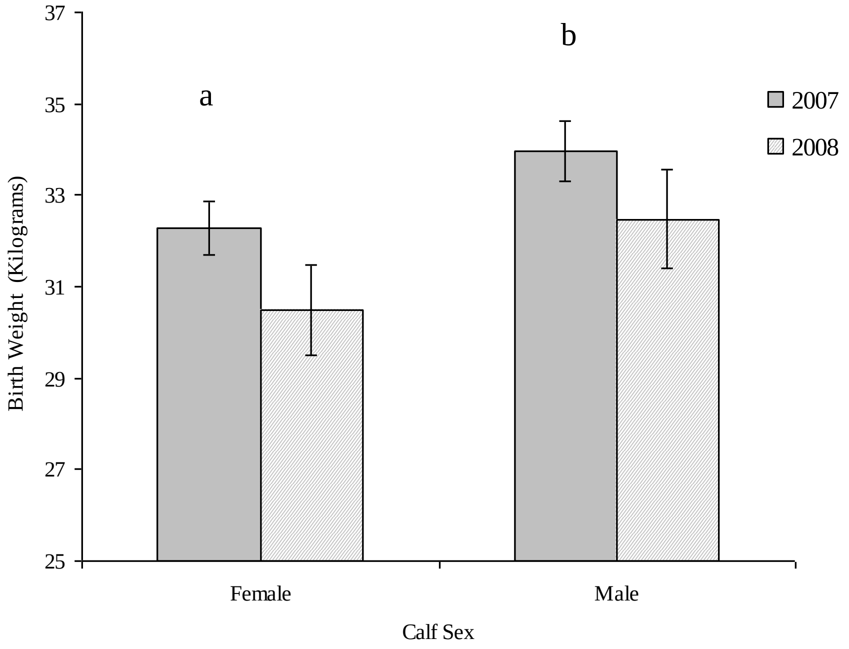
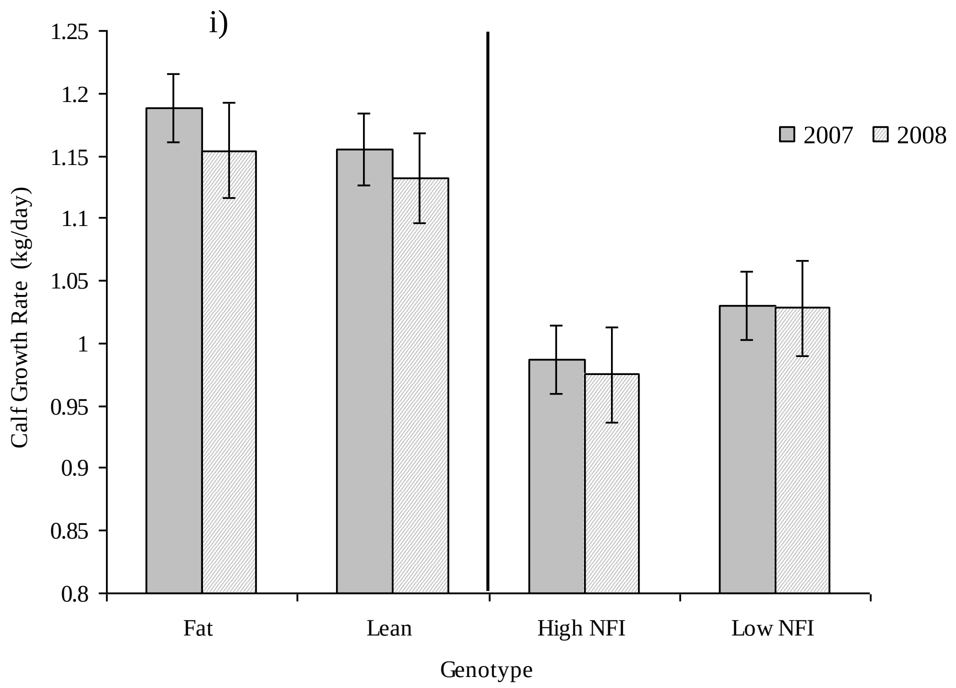


Figure 5-5: Effect of calf sex on birth weight (kg) in 2007 and 2008. Means with different letters differ significantly (P < 0.05). Error bars represent standard errors.

5.5.3 Growth rate

In 2007 and 2008 years calves of dams on the low-nutrition treatment grew more slowly than those whose dams were on high-nutrition (see Figure 5-6). Growth rate was significantly affected by height of the dam in 2007 (P = 0.05), with larger dams producing calves that grew more quickly, and by cohort in 2008 (P < 0.001) with older cows producing calves that grew significantly more quickly (P < 0.005, results not displayed). Male calves grew more quickly than female calves (2007 P = 0.067; 2008 P < 0.001, see Figure 5-7).

Significance values (P-values) and LMM model structure used for the analysis of growth rate are shown in Table 9-5 and Table 9-6.



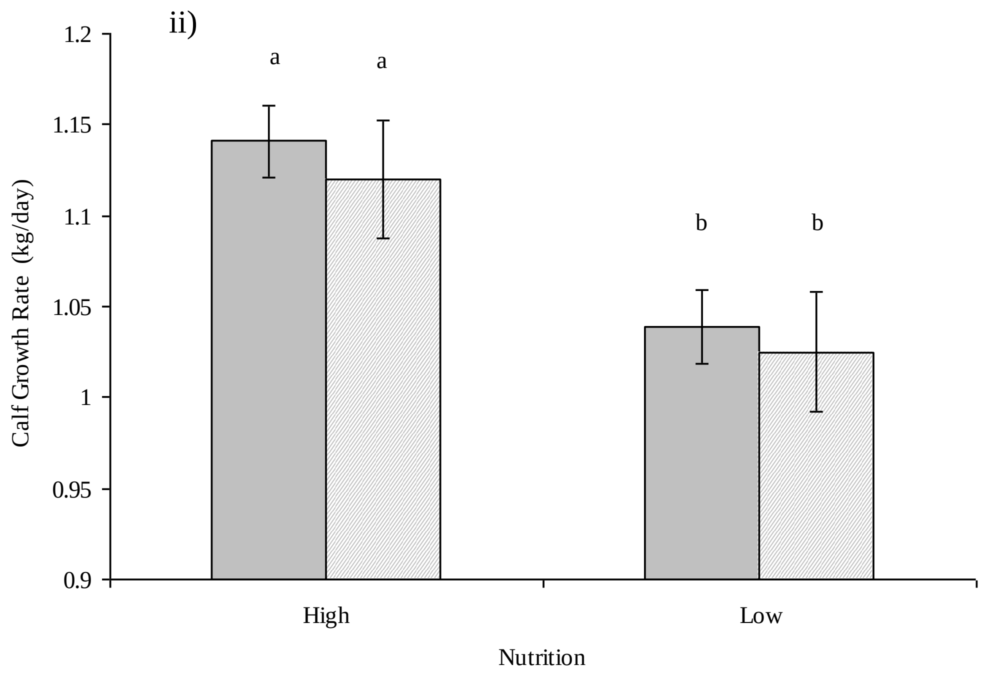


Figure 5-6: Main effect of Genotype (i) and nutrition (ii) on growth rate of calves (kg/day) in 2007 and 2008. Within line and year, means with different letters differ significantly (P < 0.05). Error bars represent standard errors.

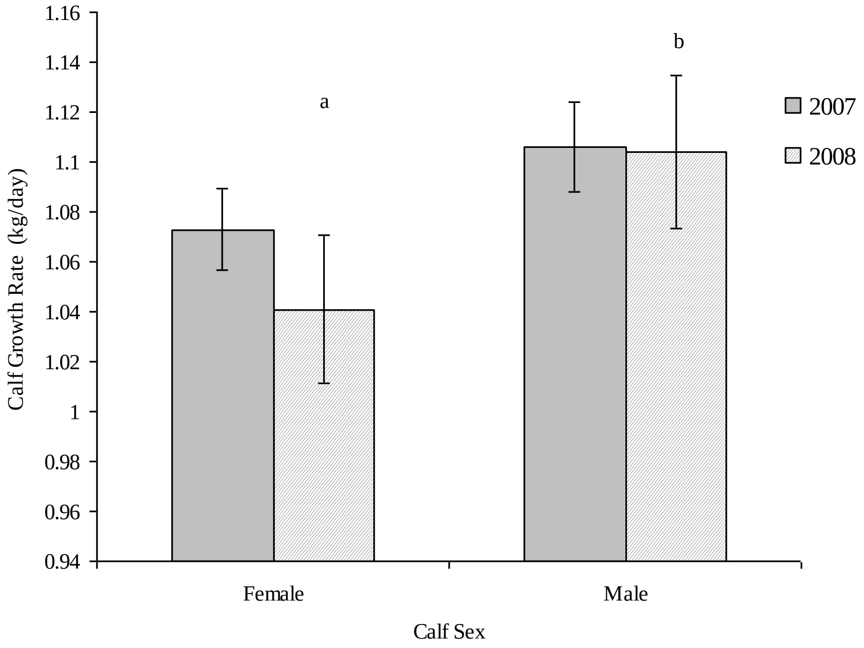


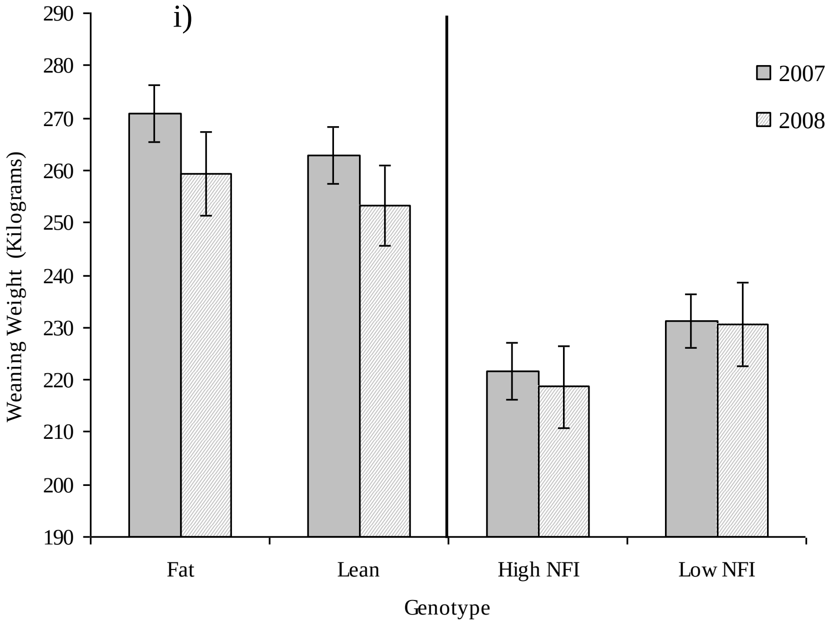
Figure 5-7: Effect of calf sex on growth rate (kg/day) of calves in 2007 and 2008. Within year, means with different letters differ significantly ($P < 0.05$). Error bars represent standard errors.

5.5.4 Weaning weight

Dams on high-nutrition weaned heavier calves in 2007 and 2008 than calves on low-nutrition ($P < 0.05$, see Figure 5-8). Male calves were significantly heavier at weaning in both years (2007 $P = 0.047$; 2008 $P < 0.001$). The height of the dam significantly ($P = 0.038$) affected weaning weight in 2007, when bigger dams weaned heavier calves ($P < 0.05$), but not in 2008 (see Table 5-1).

In both years calves that were born later in the calving season (calving date effect) were lighter at weaning ($P < 0.001$, see Table 5-1).

Significance values (P-values) and LMM model structure used for the analysis of weaning weight are shown in Table 9-5 and Table 9-6.



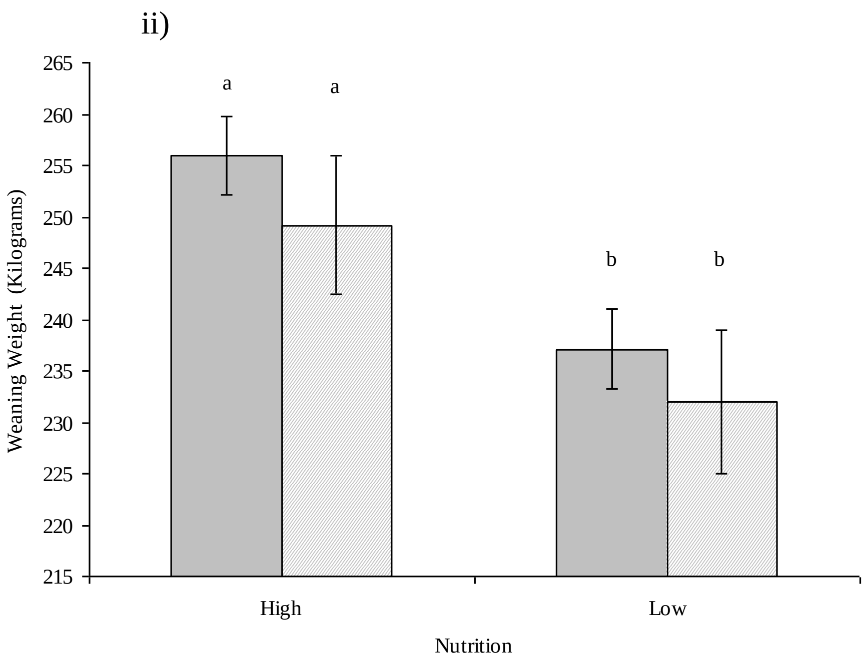


Figure 5-8: Main effects of Genotype (i) and nutrition (ii) on weaning weight (kg) of calves in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent standard errors.

5.5.5 DM disappearance, MJ ME disappearance and MJ ME disappearance per kg weaned – 2008 only

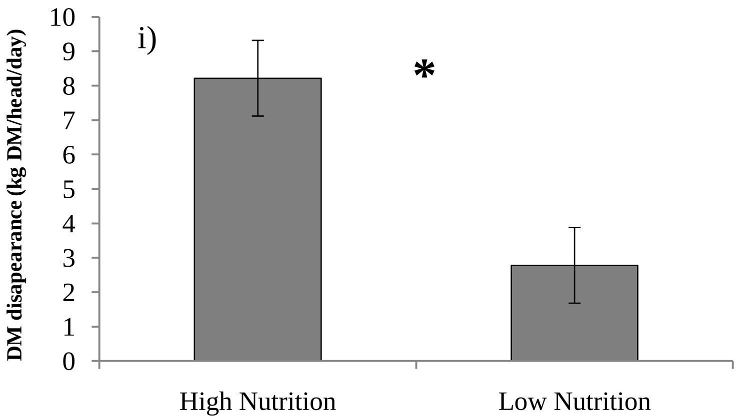
DM disappearance and MJ ME disappearance were not different between Genotypes (see Table 5-2) but were influenced by nutritional treatment (see Table 5-2). Animals on low-nutrition had lower DM disappearance, lower MJ ME disappearance and lower total MJ ME disappearance per kg weaned. There were no interactions between Genotype and nutrition treatments.

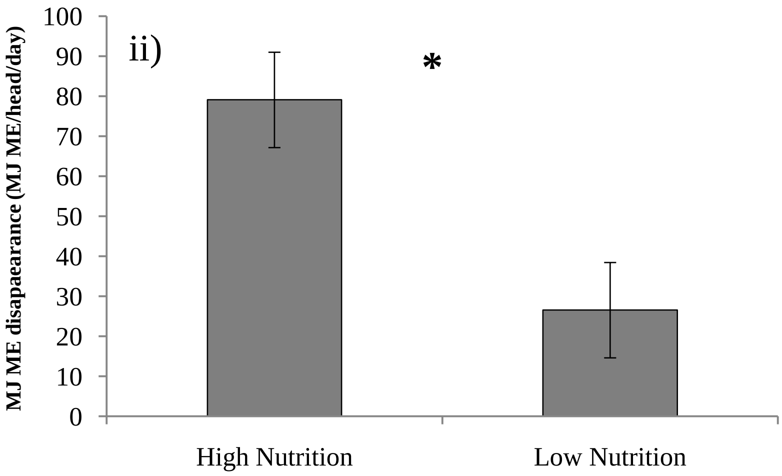
Table 5-2: Level of significance (P-values) for efficiency parameters; DM disappearance (kg DM/head/day), ME MJ disappearance (MJ ME/head/day) and MJ ME disappearance per kg weaned.

	DM disappearance	**MJ ME disappearance**	**MJ ME per kg weaned**
%lact	0.064	0.073	0.279
nutrition	**0.007**	**0.010**	**0.050**
line	0.963	0.867	0.667
HiNFIvsLoNFI	0.196	0.204	0.098
FatvsLean	0.691	0.140	0.706
nutrition.line	0.522	0.480	0.706
nutrition.HiNFIvsLoNFI	0.984	0.713	0.755
nutrition.FatvsLean	0.283	0.397	0.217

Averages for DM disappearance (kg DM disappearance/head/day), MJ ME disappearance (MJ ME disappearance/head/day), and MJ ME disappearance per kg weaned are presented by Genotype and nutritional treatment means because there was no interaction between main effects. Means of the nutritional treatments are presented in Figure 5-9 and Genotype means are presented in Table 5-3. Although the differences between Genotypes were not significant, there is a trend ($P = 0.098$) towards a difference in the MJ ME per kg weaned between the high- and low-NFI cows. High-

NFI animals had a trend towards greater DM disappearance, MJ ME disappearance, and consumed more MJ ME for each Kg beef weaned than low-NFI animals (Table 5-3). While the differences were not statistically significant (P = 0.140) there was also a numerical trend whereby Fat animals had greater DM disappearance, MJ ME disappearance, and more MJ ME disappeared for each kg beef weaned than Lean animals (Table 5-3).





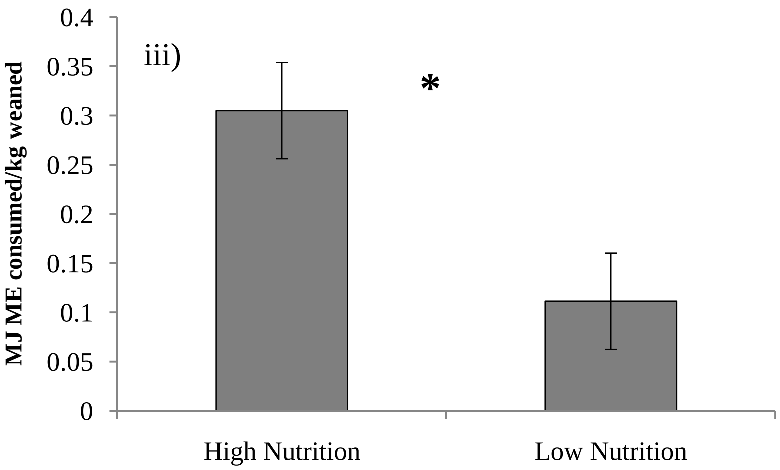


Figure 5-9: Nutrition means and SE (error bars) for i) DM disappearance (kg DM/head/day), ii) MJ ME disappearance (MJ ME/head/day) and iii) MJ ME disappearance/kg weaned**.** * denotes means are significantly different ($P < 0.05$)

Table 5-3: Genotype means (± SE) for DM disappearance (kg DM disappearance/head/day), MJ ME disappearance (MJ ME disappearance/head/day) and MJ ME disappearance/kg weaned

	DM disappearance	MJ ME disappearance	MJ ME/ kg weaned
Fat	7.1 (±1.83)	65.7 (± 19.8)	0.2 (± 0.08)
Lean	4.6 (± 1.57)	44.0 (± 17.0)	0.2 (± 0.07)
high-NFI	6.8 (± 1.60)	68.5 (± 17.3)	0.3 (± 0.07)
low-NFI	3.5 (± 1.76)	33.0 (± 19.0)	0.1 (± 0.08)

5.6 Discussion

A fundamental premise in the design of this experiment was that the nutritional treatments would result in either an over- or an under-supply of energy for all experimental animals in varying physiological states. The hurdle encountered was that the experimental design dictated that nutritional treatments included 25 animals (see Table 2-1) and nutritional intervention was triggered when an individual animal’s BCS fell below 1. This was stipulated by the University Animal Ethics Committee. When this occurred available nutrition was increased for the whole treatment until any very thin animals reached BCS 1.5. Because the average BCS across the treatment was never as low as 1, the intervention meant that nutritional treatment was often less potent than the level dictated by the design. The consequence of this was that although nutritional effects are noted in this chapter and throughout this thesis, their impacts are limited to those expected in moderate, but not extreme, nutritional restriction.

However, the conditions imposed in this experiment did closely mimic those within the beef-producing industry because it is probable that the trigger for nutritional intervention on most beef breeding enterprises would be similar to the trigger in the current experiment (McKlay, 2006, personal communication). Beef producers are generally aware that thin cattle are not productive. It is argued that any results obtained from the current experiment are very much applicable to the beef-producing community especially as the conditions mimic those in the industry.

5.6.1 Measuring DM intake on growing pasture

The use of pasture disappearance to estimate DM intake has been described before (Reeves *et al.*, 1996; Macoon *et al.*, 2003; Smit *et al.*, 2005; Meyer *et al.*, 2008) but has been shown to be difficult and to yield variable results. Other methods of measuring pasture intake do exist such as the use of intra-ruminal alkane capsules (Dove and Mayes, 1991; Reeves *et al.*, 1996; Macoon *et al.*, 2003; Valiente *et al.*, 2003; Smit *et al.*, 2005) but this method is expensive and difficult to interpret. The methodology used in the current experiment was considered to be the only practical method available to the author to obtain an estimate of DM disappearance in 16 replicate groups on a 200 hectare grazing trial. The methodology was designed to identify differences in DM disappearance but was not sufficiently precise to identify the differences in intake between Genotypes that have been identified in concentrate-fed animals. These results will be discussed later in the chapter.

5.6.2 Production parameters

The discussion pertaining to production parameters is restricted to the results of DTC, and birth, growth and weaning weights of calves. The impact of nutrition on DTC in 2008 but not 2007 was attributable to a couple of factors. Firstly, in young, primiparous cattle DTC is a sum of the effects of age at first puberty, weight and BCS, sire, and year and month of joining (Johnston and Bunter, 1996). It is also influenced by the variation in gestation length, because the measurement period ends when the cow calves. As a consequence, the major drivers of DTC in primiparous cattle are the regulators of sexual maturity and fertility. This is supported by the results from this experiment that show an effect of dam birth date on DTC (see Table 5-1) in 2007 but not 2008. In 2007 all cows were primiparous and subjected to the influences of the timing of sexual maturation, but in 2008 the majority of the herd was multiparous and these effects are somewhat diluted. The effect of dam age on DTC is supported by Johnston and Bunter (1996).

Secondly the timing of the imposition of the nutritional treatments was important. There is no doubt that nutrition has a large role to play in the processes of reaching sexual maturity and ovulation of fertile oocytes (Foster and Olster, 1985; Schillo *et al.*, 1992; Zieba *et al.*, 2004; Yilmaz *et al.*, 2006; Martin *et al.*, 2007). The experimental design was such that the allocation of the animals to the nutritional treatments was done using selected pregnant animals in their last trimester of pregnancy (see Section 2.3). Until that time there had been no nutritional restriction imposed on the animals, which had been provided with nutrition above that required for the maintenance of young breeding cattle. It is therefore unsurprising that no nutrition effects were noted in 2007.

In 2008 the nutritional effect was notable, with cows on low-nutrition having a longer DTC. This effect was noted across all experimental Genotypes. Restricted nutrition will extend the period of ovarian inactivity, produce fewer fertile ovulations and retard follicle development (Lucy *et al.*, 1991; Adams *et al.*, 1992; Ginther *et al.*, 1996; Beam and Butler, 1997; Gutierrez *et al.*, 1997; Crowe *et al.*, 1998; Mackey *et al.*, 2000; Diskin *et al.*, 2003; Webb *et al.*, 2004; Crowe, 2008) and ultimately increase the PPAI (Wiltbank *et al.*, 1962; Richards *et al.*, 1986; Wright *et al.*, 1992). In this experiment there was a significant difference in fatness in both years during the breeding season (see Figure 3-4). Greater BCS at the time of calving have been shown to cause an increase of the PPAI (Richards *et al.*, 1986; Osoro and Wright, 1992; Wright *et al.*, 1992; Rhodes *et al.*, 2003), and pregnancy rates in multiparous cows are also affected. The combination of the effects of restricted nutrition on follicle initiation, development and maturation, and of decreased BCS leading to an increase in PPAI, leads to the increase in DTC that was seen in this experiment in cows on the low-nutrition treatment.

DTC results were most influenced by the period when the animals were finishing calving and entering the joining period. It is even more notable that this difference was evident because as mentioned in section 2.12, the nutritional restriction was somewhat disturbed in 2007 by unseasonal spring flush. Despite this moderated nutritional treatment the difference in DTC was still evident, suggesting that although the nutritional treatments converged for a month in 2007, the average difference was still great enough to impact on the parameters that affect DTC.

The lack of an impact of Genotype on DTC was consistent in both years of the experiment. Arthur *et al.* (2005) reported a trend towards low-NFI cattle calving later than high-NFI cattle, and suggested that this be monitored in the future because of the tendency for more efficient individuals in other species to have mild reproductive impairments. So far no investigations into the association between NFI and sexual maturation in cattle have been published. The results from this experiment do not identify a difference in female fertility through either the measurement of DTC, or the estimate of PPAI via the measurement of blood progesterone (see Chapter 4). These results are not particularly sensitive and it is possible that with the addition of the results of other, similar experiments, such as those being conducted at Struan, South Australia, reproductive consequences of selection for NFI may be identified.

Fat cows were fatter than Lean cows during the breeding season in both years (see Section 3.5.3) and the hypothesis was that the relationship between BCS and DTC would result in a Genotype difference in DTC. This did not happen and is probably because there were no extremes of BCS during the breeding season. Mean P8 measurements during the breeding season were 11.5mm fat for Lean cows. Graham (2006) suggests that a measure of 8-12mm fat at the P8 site translates to a BCS of 2.5. For there to be an impact of BCS on DTC it would be expected that BCS would be 2 or lower (Richards *et al.*, 1986; Wright *et al.*, 1992; Ciccioli *et al.*, 2003), so the leanness of the cows in this experiment was not enough to affect DTC. It was also found that there was an increase in fatness in all Genotypes post calving (see Figure 3-5), and a positive change in BCS post-calving is associated with increased fertility (Rutter and Randel, 1984; Richards *et al.*, 1989; Vizcarra *et al.*, 1998; Reist *et al.*, 2000; Lake *et al.*, 2005), further contributing to the similarity in DTC between Genotypes.

The analysis of DTC is not particularly powerful in the current experimental design. Power calculations based on farm records over a five year period suggest that there is a 90% chance of detecting a 10% difference in DTC at the 5% significance level using 25 cows per treatment. For a DTC of 315 days (the average of the two years of the experiment), this would mean that only a difference of 31.5 DTC or greater would be detectable in the analysis. This equates to greater than a full oestrous cycle difference between Genotypes. Although there was no detectable difference in DTC between Genotypes, there may be an undetectable difference of fewer than 31.5 days. In 2008 the DTC for fat animals was 5.6 days shorter than for lean animals while in 2007 it was 2.6 days longer. In 2008 the DTC for high-NFI animals was 0.9 days longer than for low-NFI animals, and in 2007 it was 3.5 days shorter. These differences would probably increase if the experiment could be repeated for more than just the two parities, and become significant as the cows matured. Cow longevity is an important contributor to MP and one that cannot be estimated in this experiment. The increase in DTC over time that may occur if differences, albeit small, do exist between Genotypes, would be a key parameter in evaluating cow longevity. This is worth assessing in future research. It must again be noted that the experiment is being replicated in South Australia and will continue for two more years so this will likely add this extra data.

The impact of nutrition on DTC reinforces the long established principle that adequate levels of nutrition during the post-partum period are essential to maintain a 365-day breeding cycle in beef cattle. Fatness and BCS of the animals are a reflection of nutritional history and although important in the provision of energy during high energy demand period such as lactation, are less of an influence on DTC than energy supply through intake during the rebreeding period.

The absence of any effect of Genotype or nutrition on the birth weight of calves in either year of the experiment is not unexpected. It has been reported that restricted nutrition before calving has an impact on the birth weight of calves (Bellows and Short, 1978; Wiltbank and Remmenga, 1982; Pleasants and Barton, 1992; Spitzer *et al.*, 1995; Cafe *et al.*, 2006) but some report that prepartum level of nutrition has no effect on calf birth weights (Bellows *et al.*, 1982; Lake *et al.*, 2006). It is likely that because the nutritional treatment was only imposed in the last trimester of pregnancy before the 2007 calving period, nutrition would have no effect on birth weights in that year. Before the 2008 calving, although the nutritional treatments were in place, for reasons already defined, the energy restriction was possibly not extreme enough to induce the differences in birth weights reported by other researchers. Low-nutrition cows calved in on average BCS 2, suggesting a history of inadequate but not extremely low-nutrition. Regardless of the impact of nutrition, more importantly there was no effect of Genotype on birth weights. In 2008 there was a difference between the lines of cattle (Industry vs. NFI – data not presented) but this is explained by the significant difference in the size of animals in the two lines. Industry animals are taller and heavier than NFI animals, which had had no selection pressure for growth or muscularity or fatness for several generations. The focus at the breeding centre in Trangie was solely to produce cattle with divergent NFI. In terms of the relevance to beef producers of this finding, the conclusion based on the results of this experiment is that there is no risk of compromising birth weights with selection for leaner or more highly efficient cattle.

The important finding regarding the growth rate and weaning weight of calves was that they were not affected by Genotype. Selection for leanness or feed efficiency did not impact on these parameters. This result is supported by studies that show that cows from low- and high-NFI classifications have similar milk yield, when determined by the weigh-suckle-weigh method (Arthur *et al.*, 1999) which translates to similar calf growth rates. In support of the results from this experiment, Herd *et al.* (1998) and Arthur *et al.* (2005) found that calves from low- and high-NFI dams had similar growth rates and weaning weights. However, the significant effect of nutrition suggests that energy supply does influence the output from the production system. Calves of cows on low-nutrition grew notably more slowly than high-nutrition calves, a relationship that highlights the decreased milk production in cows on restricted nutrition (Roche *et al.*, 2000; Meikle *et al.*, 2004; Cafe *et al.*, 2006). Calves with higher weaning weights tend to be from dams with greater levels of milk production (Davis *et al.*, 1983).

The effect of cohort (see Table 5-1) has been reported in other studies which suggest that primiparous cattle had lower BCS and produced less milk post partum than multiparous animals (Meikle *et al.*, 2004), contributing to a slower growth rate of the calves. Third trimester P8 fat measure as a reflection of pre-calving BCS was not associated with growth or weaning weight in either year of the experiment. This again supports the hypothesis that energy supply after calving rather than energy reserves pre-calving are the important factor for the output of the production system in terms of growth and weaning weights. Calves grow faster and wean heavier when their dams are fed better, regardless of the BCS of the dam pre-calving. Producers should recognise the importance of adequate post-calving nutrition on the profitability of the production system in managing cow fertility as well as the kg beef output, but be reassured that selection for greater feed efficiency or leanness does not compromise these important production parameters.

5.6.3 Efficiency parameters

DM disappearance and MJ ME disappearance

With feed being one of the highest input costs in the production system it is an appealing concept for producers to be able to select animals that eat less feed for the same level of production. This experiment has already shown that there was no difference measured between the Genotypes in the production parameters of DTC, birth weights, growth rates and weaning weights of calves, so measuring the feed input into the different Genotypes was important in assessing whether there is true benefit in selecting for leanness or feed efficiency.

Measuring DM intake in cows selected for a divergence in feed efficiency has been reported in only two studies (Herd *et al.*, 1998; Meyer *et al.*, 2008). The first by Herd *et al.* (1998) reported that the differences in intake between cows selected for a divergence of feed efficiency, identified in young cattle on concentrate diets, persist in the same cows when they are mature and grazing pasture. The other study by Meyer *et al.* (2008) concluded that either no intake differences existed between low- and high-NFI cows grazing pasture, or that current methodology and small animal numbers in their experiment limited the ability to detect differences. They postulated that similar grazed forage intake results observed between low- and high-NFI cows may have occurred because the animals they tested were in a different physiological state to those studied by Herd *et al.* (1998). The demands of gestation and lactation may have blunted any NFI differences seen during growth (Meyer *et al.*, 2008).

Research that examines the response in terms of growth rate and feed conversion ratio of high- or low-NFI animals on pasture is more readily available. Herd *et al.* (2002b) showed that steers grazing pasture had different feed efficiency according to the NFI of their dams because there was a positive regression coefficient for feed conversion ratio with mid-parent EBV for NFI (2.9; $P < 0.1$). In another study it was shown that when restriction of pasture availability limited the growth rate of steers, those selected for low-NFI grew faster (Herd *et al.*, 2005). These two studies suggest that the particular feed efficiency of animals measured in a feedlot will persist in a grazing situation. It also appears that animals bred from highly feed-efficient cows will inherit this increased feed efficiency and it will be evident when they graze green pasture.

The current experiment did not identify statistically significant differences in DM disappearance between high- and low-NFI cattle, although there is a numerical difference of 50.8% between the mean DM disappearance of high- vs. low-NFI Genotypes, with low-NFI cattle appearing to eat half as much as high-NFI cattle. The probable reason for the lack of statistical significance was that the variation in DM disappearance results between replicates is too high. This was not unexpected given the difficulty of measuring DM disappearance on growing pastures. Each treatment was only replicated twice in this experiment and an increase in the number of replicate groups would probably have improved the power of the analysis, so it is postulated that an experiment with a greater number of replicates may have overcome this problem and the implied differences in intake would become statistical realities. This was beyond the capabilities of the current experiment because of financial and spatial constraints as well as the difficulty in sourcing appropriate genetically divergent animals. It is entirely possible that the difference in feed intake is actually real given that there is only a 19% probability (high- vs. low-NFI DM disappearance $P = 0.196$ see Table 5-2) that if differences are identified there are in fact no differences at all. This result indicates a trend towards low-NFI cows eating less green pasture than high-NFI cows.

An experiment is currently underway which replicates the current experimental design on another site (Struan, South Australia) where there are three replicates for each Genotype. The results of the current experiment will ultimately be combined with the results of the Struan experiment to add statistical power to the analysis of DM disappearance. It is hypothesised that the “addition” of three extra replicate groups may overcome the impact of between-replicate group variation and the differences will become significant.

Similarly there is no statistically significant difference in DM disappearance, and therefore MJ ME disappearance, in pasture grazed by Fat or Lean cows, despite the numerical difference of 35% in favour of Lean cows (see Table 5-3). The same conclusions regarding measurement of intake and lack of statistical power are applicable for this result. It was hypothesised that Fat animals would eat less DM and consume fewer MJ ME than Lean animals owing to their lower maintenance requirements (DiCostanzo *et al.*, 1990; DiCostanzo *et al.*, 1991; Egan *et al.*, 2001; Walmsley and Parnell, 2009) but this has not been identified, and there was actually a trend to the reverse with Fat animals apparently tending to eat more than the Lean. The likely reason for the numerical difference is either that there was no real difference at all, or Fat animals simply ate more of the available feed and became fatter due to their genetic predisposition towards fatness. The Fat animals did accrue fat faster than Lean animals. Perhaps the genetic potential was driving greater intakes and therefore increased fatness in the Fat animals. This explanation is supported because when pre-calving fatness is used as a covariate at the top of the statistical model, significance levels for DM disappearance differences become markedly less significant (results not presented). The reason for excluding this covariate from the final model was that it was not desirable to correct for the very factor that selection of animals to the experiment was based upon. Further work with more animals and replicate groups is required to determine statistical and biological significance of the trends identified here.

The significant difference in DM disappearance between the nutritional treatments indicates that the experiment worked in terms of restricting energy supply to the animals on the low-nutrition treatment. Across all Genotypes low-nutrition animals had lower DM disappearance measures than high-nutrition animals (see Figure 5-9). This result confirms that in the 2008 grazing season the nutritional imposition resulted in an actual difference in MJ ME consumed between high- and low-nutrition treatments. Because of the difficulty in measuring pasture disappearance, and the exclusion of estimates of pasture growth rates in the model, it is not possible to generate actual measures of energy input. This would have been useful when calculating the extent to which there was a deficit in energy supply on the low-nutrition treatment, and how far below maintenance energy requirements the treatment was. More intensive, individual animal studies would be useful to answer these questions. The conclusion reached was that measurement methods of pasture disappearance were not precise, and that there was great difficulty in reproducing results in several replicates, the same conclusions reached by Meyer *et al*. (2008) in research that was published two years after the beginning of the current experiment.

MJ ME consumed per kg beef weaned.

This measure is a true "efficiency parameter" as it takes into account both inputs and outputs and is very similar to the cow/calf efficiency parameter (Jenkins and Ferrell, 1994). Cow/calf efficiency is closest to the most common efficiency measure, namely GF. GF is simply the ratio between production outputs and feed inputs in the whole production system (Archer *et al*., 1999). Calculation of cow/calf efficiency involves measuring the total feed intake of the cow and her progeny over an entire production cycle. This is usually done with multiparous cows, from weaning of one calf to weaning of the next calf. The total feed intake over the production cycle is then compared with the weight of calf weaned to express the efficiency of the cow/calf unit in terms of kg calf weaned per kg feed eaten (Archer *et al.*, 1999).

Cow/calf efficiency does not take into account the change in liveweight of the dams over the year, nor the number of MJ ME that are contained within each kg DM eaten over time. In this experiment both of these have been accounted for, but the efficiency estimate was not over a full production cycle, but rather confined to the green pasture grazing season. The reasons for this are firstly that feed efficiency differences of cows eating concentrated feed have already been reported, including in the experimental Genotypes of high- and low-NFI cattle when they underwent an individual feed test (see Section 2.2). The parameter of *MJ ME consumed per kg beef weaned* also does not take into account feed inputs of the slaughter generation from weaning to slaughter, but it has been reported that feed used for post-weaning growth is a relatively small part of the total feed used in a cow/calf production enterprise (Gregory, 1972) and the parameter is still probably reflective of the efficiency of the whole system.

Secondly, the main focus of this experiment was to identify efficiency differences between Genotypes on extensive grazing systems and thus the experiment was restricted to times when animals were grazing. However, it is noted that most beef production enterprises in South West Western Australia do have a period of supplementary feeding their cows. This is often in late summer when dry feed does not meet the energy and protein demands of pregnant cows and the growing slaughter generation (MLA, 2004). It can be argued that a robust efficiency parameter would include this period, but the difficulty and resource expense in managing measurement of this was too great for this experiment. Animals were grouped according to nutritional treatment and Genotype, and there was no accurate means of measuring supplement intake during the supplementary feeding period. As a consequence the efficiency parameters are specific to the nine months from start of calving to weaning when the animals are grazing. It is postulated that efficiency parameters during this period will be reflective of the efficiency of the animals over the entire production cycle.

It was demonstrated that cows on the low-nutrition treatment ate fewer MJ ME/kg beef weaned (see Figure 5-9) which is interesting because at face value this suggests that feeding less is potentially more profitable. It is postulated that rather than this the result means that on a plane of high-nutrition, such as was imposed on the cows in this experiment, energy input is not fully translated to a production output. The difference in fatness between nutritional treatments shows that when energy is supplied in excess it is stored as fat, and although there was a difference in weaning weight in the high-nutrition treatment, most of the excess energy was stored as fat. This result indicates that a balance must be struck between increasing productivity by increasing stocking rates, and limiting the body energy reserves through too great a restriction in energy supply.

This experiment has shown that one year of energy restriction has a significant impact on DTC, a problem that would no doubt be compounded after several generations of energy restriction. The perceived benefits of dramatically increasing stocking rates would probably be neutralised by compromised fertility in the long term. The result does suggest that the critical level of nutrition for productivity to remain high may well be lower than the high-nutrition level in this experiment but higher than for the cows on low-nutrition, and supports the conclusion that maintaining a BCS of 3-3.5 in breeding cows translates to the highest productivity interval (Richards *et al.*, 1986; Osoro and Wright, 1992; Wright *et al.*, 1992; Rhodes *et al.*, 2003; Meikle *et al.*, 2004). The result needs to be considered in the wider context of the whole production system including factors such as stocking rates adjusted to suit FOO, size of grazing area, breed and age of cows and target markets.

With the results of this experiment identifying no difference in MJ ME consumed per kg of beef weaned (see Table 9-7) it can be concluded that there was no difference in efficiency between the Genotypes. There was no difference in weaning weights between Genotypes (see Section 5.5.4) as well as no difference in MJ ME disappearance (see Section 5.5.5). This result has important implications in terms of the evaluation of MP. Although there was no positive association between selection for greater feed efficiency or leanness and efficiency parameters, it is argued that the absence of a negative effect is the more important finding. With no adverse impact on MP evident from the selection for more feed-efficient or leaner animals, beef producers can be confident that if these traits are desirable and used for selection in their enterprise, MP will not be compromised. Birth weights were similar, meaning no more or fewer incidents of dystocia and no compromise on final finishing weight. Growth and weaning weights were similar suggesting no compromise in productivity during the pre-weaning phase. Intake of energy was also similar, suggesting no greater or lesser requirement for feed in one Genotype or another. These production and efficiency measures were encapsulated in the *MJ ME consumed/kg beef weaned* parameter. Together they provided quantifiable measures of MP, and address the concerns among producers about the perceived risks of selection for feed efficiency or leanness.

However, if the trend (see Table 5-3) towards low-NFI animals consuming fewer MJ ME/kg weaned that was found in this experiment was real, this would have even more relevance to the beef industry. It is hypothesised that for reasons explained previously, the limited number of replicates and the difficulty in measuring intake on grazing pasture led to the lack of statistical significance when analysing efficiency parameters. Indications are that with more replicates, and more precise measures of intake, these trends may be shown to be real. This would imply that not only do more feed-efficient cows eat less, they eat fewer MJ ME per kg weaned, making them more productive than their high-NFI counterparts. This finding, although not documented in this work, would be of great importance to the industry and would result in NFI being considered a very important production trait for not just the finishing phase of production but for cow/calf enterprises as well. It would result in significant increases in producer profitability through increased stocking rates and increased turnout of beef in the form of the slaughter generation. The result was similarly present in the comparison between Fat and Lean cows but is less pronounced. Regardless of whether the trends in the results are accepted as real or possible, it is clear that MP is not compromised when there is selection for leanness or feed efficiency.

The second part of the major hypothesis in this chapter is that low-NFI or Lean animals would lose their perceived economic benefit over Fat or high-NFI animals when energy input was restricted. This was not the case (see Figure 5-9). Although nutritional treatment did impact on efficiency parameters there was no interaction with Genotype. No one Genotype performed worse under the low-nutrition treatment. This is another important industry finding. It suggests that the pressure of reduced energy supply does not lead to a compromise in MP in animals that have been selected for what are considered the economically beneficial traits of feed efficiency and leanness. Earlier results confirm that Lean cows and low-NFI cows yield more meat than Fat or high-NFI cows (see Section 3.5.5), which translates to increased profitability for the beef producer. This experiment has shown that although there was no identifiable difference in intake and MJ ME consumed/kg beef weaned between Fat and Lean animals, it is still more desirable to breed leaner animals because of the recognised benefits of breeding higher-yielding cattle.

5.7 Conclusions

This chapter highlights through measurement of production and efficiency parameters in grazing beef cows that MP is not compromised by selection for greater feed efficiency or leanness, and that the economic benefits of selection for these traits persist in the energy restricted environments tested in this experiment. There are no impacts on fertility, pre-weaning parameters or efficiency indices evident when selecting for feed efficiency or leanness. Indications were in fact that there were trends to suggest that Lean and low-NFI cattle perform better than their Fat and high-NFI counterparts in terms of production efficiency and these results need to be replicated and validated in other experiments.

CHAPTER 6. BIOCHEMICAL, ENDOCRINE AND PHYSIOLOGICAL RESPONSES TO RESTRICTED NUTRITION IN COWS SELECTED FOR A DIVERGENCE IN FATNESS OR FEED EFFICIENCY

6.1 Introduction

Feed costs have a significant impact on the profitability of a beef production system. Providing feed is one of the largest inputs, and the opportunity to select animals that consume less feed is a concept that has much appeal to the beef-producing community. It was estimated by the United States Department of Agriculture that in 2004 and 2005 feed-associated costs made up 50.2% and 55.7%, respectively, of all non-fixed costs in US cow/calf operations (ERS, 2005). As such, identifying NFI as a heritable trait (Crews, 2005) has provided potential for improved economic benefit. It is also now accepted that less feed-efficient animals have a greater environmental impact, largely through increased methane emissions (Nkrumah *et al.*, 2006), making selecting for more feed-efficient animals an attractive option in times when carbon footprints of different industries are being closely examined by governments worldwide.

The biological basis for a difference in NFI has been discussed in three papers (Richardson and Herd, 2004; Richardson *et al.*, 2004; Herd and Arthur, 2008). These authors identify five processes that contribute to the difference in net feed efficiency, variation in namely feed intake, digestion of feed, metabolism (anabolism and catabolism associated with and including variation in body composition), physical activity and thermoregulation. Individually the first four processes each account for only a small percentage (between 5% and 14%) of the variation but together were responsible for one third of the variation in NFI. The remaining two thirds are accounted for by differences in the processes that result in heat production and ultimately heat loss through evaporation. These processes include ion transport, protein turnover, tissue metabolism, and the biological effects of stress. The physiological mechanisms identified so far are based on only a few studies, some of which have a small sample size. The exact differences in the physiological processes have proved difficult to elucidate and explain and further research needs to be done in this area. This experiment focuses on the processes involved in the distribution of energy and its association with body composition and metabolism.

The distribution of energy consumed in a production system is a driver of profitability. Of all physiological processes the deposition of fat is one of the most energy expensive (Murray *et al.*, 1988). Increased adiposity is associated with decreased feed efficiency (Herd and Bishop, 2000; Arthur *et al.*, 2001; Nkrumah *et al.*, 2004a; Arthur *et al.*, 2005) and it also impacts significantly on reproduction (Richards *et al.*, 1986; Osoro and Wright, 1992; Wright *et al.*, 1992; Rhodes *et al.*, 2003). Greater body energy reserves on the whole result in a more fertile animal that conceives sooner and more readily than leaner animals. However, there are distinct benefits to producers in selecting for leanness in cattle, particularly because of the association with increased meat yield (Wolcott *et al.*, 2001; Nkrumah *et al.*, 2004a; Rourke *et al.*, 2009). Selecting for increased feed efficiency or leanness is effectively selecting animals whose physiology is somehow different from those less feed-efficient or fatter animals (Richardson and Herd, 2004; Richardson *et al.*, 2004; Stevenson, 2007; Herd and Arthur, 2008). This chapter aims to determine if these differences are apparent in the measurement of circulating blood parameters, and if apparent to discuss their impact and significance to producers of beef.

The breeding cow experiences major changes in energy metabolism, energy balance, and feed intake during the reproductive cycle. Energy demands vary greatly between the first trimester of pregnancy and the immediate postpartum period. These changes are reflected in the energy metabolites and circulating hormones (blood parameters) which act as regulators of energy metabolism and reproductive function. The relationships between blood parameters and fatness, level of nutrition, reproductive performance, energy balance in beef cattle have been explored extensively (Rutter *et al.*, 1989; Spicer *et al.*, 1990; Rutter and Manns, 1991; Etherton *et al.*, 1993; Spicer *et al.*, 1993; Barash *et al.*, 1996; Yelich *et al.*, 1996; Roberts *et al.*, 1997; Stick *et al.*, 1998; Delavaud *et al.*, 2000; Reist *et al.*, 2000; Block *et al.*, 2001; Ehrhardt *et al.*, 2001; Delavaud *et al.*, 2002; Johnston *et al.*, 2002; Zulu *et al.*, 2002; Liefers *et al.*, 2003b; Konigsson *et al.*, 2008; Oikonomou *et al.*, 2008; Kelly *et al.*, 2010) but there has been little exploration of the association in relation to a divergence in feed efficiency or yield.

Attempts have been made in the past to associate differences in measured blood parameters with genotype variation in order to use them in marker-assisted selection (MAS) (Stick *et al.*, 1998; Richardson *et al.*, 2004; Wood *et al.*, 2004; Moore *et al.*, 2005), but with limited success (Johnston, 2007; Kelly *et al.*, 2010). This chapter explores the associations between circulating blood parameters and phenotypes in beef cattle selected for a divergence in either feed efficiency or fatness over two breeding seasons, and investigates the possibility of using circulating blood parameters as a tool for MAS. It also examines the possibility of using particular blood parameters as markers for fertility.

6.2 Aims

The aims of this experiment were to:

1. characterise the concentrations of blood parameters, pre- and post-calving, in beef cows on two levels of nutrition and of differing genotypes under grazing conditions. Specific measured blood parameters were: IGF-1, GH, insulin, leptin, BHB, glucose, NEFA and acetate;
2. identify any association between circulating concentrations of blood parameters and Genotype of the experimental animals;
3. investigate the impact of two levels of nutrition on circulating concentrations of blood parameters, pre- and post-calving, in two successive breeding seasons;
4. associate circulating measured blood parameters with fatness or feed efficiency, and to identify any parameters whose fluctuations correspond closely with production or efficiency measures;
5. investigate the association between circulating concentrations of blood parameters and MP in different genotypes of cattle.

6.3 Hypotheses

It was hypothesised that:

1. nutritional treatment, rather than Genotype, would affect pre- and post-calving measures of blood parameters;
2. where differences between Genotypes exist, they would be explained by differences in energy balance and/ or the adiposity of the animals;
3. there would be no single blood parameter that would represent a physiological marker for MP

4. it would not be possible to use blood parameters for MAS in the selection of feed-efficient animals.

6.4 Materials and methods

6.4.1 Animals

The animals used in this experiment are as described in Section 2.1. Table 2-1 outlines the experimental design and allocation of animals to the experiment. In 2007 only the 1st cohort of animals had calved and therefore the data in this year was sourced from the 1st cohort only, whilst in 2008 both cohorts were included in the data set.

6.4.2 Data collection

Animal data

Blood samples were collected from all animals in the last trimester of pregnancy and then during the post-calving period. The technique for collecting blood samples is described in Section 2.6 and the frequency of sample collection is described in section 2.9.3. The post-calving blood sampling period ended one week before the 2007 joining period and in 2008 ended at the end of the joining period. The period of blood sample collection in 2008 was extended to obtain measures of progesterone concentrations up to the end of the joining period in an attempt to quantify the PPAI of the different Genotypes, as described in Chapter 4.

Measurement of fatness.

All experimental animals had US measures of fat taken during a two week period in the middle of the calving period in 2007. Measurements were taken more frequently in 2008 with US scans conducted monthly in 2008 from May to December.

This period included the 3rd trimester of pregnancy through until the end of the joining season. As described in Section 3.4, the scanning resulted in a measurement of fat depth at the Position 8 (P8) site.

Pasture data

Pasture data was collected during the growing season as described in Chapter 5.4.3.

6.4.3 Statistical Analysis

For a general description of statistical analyses and an explanation of fixed and random terms in the models described below see Section 2.10.3.

Pre-calving blood parameter analysis

An LMM with the following fixed and random effects was used to identify significant effects of Genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition and their interactions on a single measurement of biochemical and endocrine parameters from dams, taken in the last trimester of pregnancy in 2007 and 2008.

Fixed model:

constant + location + line + cohort (2008 analysis only) + height + FatvsLean + HiNFIvsLoNFI + calving date + 3rd trimester P8 + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition

Random model:

replicate group + dam ident

The effects of genotype within line and nutrition were corrected for location and cohort (2008 only). In addition, the main effect of nutrition and interactions between genotype and nutrition were corrected for the 3rd trimester P8.

Post-calving blood parameter analysis

Statistical analysis was used to identify significant effects of Genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition and their interactions on concentrations of blood parameters, measured from dams at regular intervals after calving. Each animal had up to eight blood samples collected from it but calving date restricted the number of samples collected, such that later-calving cows had fewer samples collected from them. Examination of individual-animal results plotted against time revealed no indication of a curvilinear trend over time to the results; rather there was on average a linear trend in levels of blood parameters from calving to the end of the breeding season. A linear effect of days post-calving (day-post-calving) in the random coefficient model was therefore justified. A LMM with the following fixed and random effects was used to identify significant effects of Genotype (line, FatvsLean, HiNFIvsLoNFI), nutrition, day-post-calving and their interactions biochemical and endocrine parameters in 2007 and 2008:

Fixed model:

constant + location + lactating + calving date + line + cohort (2008 analysis only) + height +3rd trimester P8 + FatvsLean + HiNFIvsLoNFI + nutrition + line.nutrition + FatvsLean.nutrition + HiNFIvsLoNFI.nutrition + pre-calving measure + days-post-calving + days-post-calving.nutrition + days-post-calving.line + days-post-calving.FatvsLean + days-post-calving.HiNFIvsLoNFI + days-post-calving.line.nutrition + days-post-calving.FatvsLean.nutrition + days-post-calving.HiNFIvsLoNFI.nutrition

Random model:

replicate group + replicate group. days-post-calving + dam ident + days-post-calving. dam ident

Main effects of Genotype within line and nutrition and interactions were corrected for location, whether a cow was lactating or not, the calving date, the height of the animal and a measure of P8 fat in the last trimester.

Rate of change of blood parameter compared to changing fatness

The rate of change of measured blood parameters was analysed to examine the relationship between the post-calving rate of change of each measured blood parameter and the rate of change of P8 fatness over the calving period. For each parameter that was measured post-calving, a regression was fitted to produce a slope and therefore a rate of change over time. Each slope then became the y-variate in a LMM model which was used to identify significant effects of Genotype and nutrition on the relationship between parameter change and change in fatness. The LMM had the following fixed and random model:

Fixed model:

constant + *location* + *cohort* + *line* + *height* + *FatvsLean* + *HiNFIvsLoNFI* + *calving date* + *nutrition* + *line.nutrition* + *FatvsLean.nutrition* + *HiNFIvsLoNFI.nutrition* + *P8slope* + *location.P8slope* + *cohort.P8slope* + *line.P8slope* + *heightJan2008.P8slope* + *FatvsLean.P8slope* + *HiNFIvsLoNFI.P8slope* + *calvingdate.P8slope* + *nutrition.P8slope* + *line.nutrition.P8slope* + *FatvsLean.nutrition.P8slope* + *HiNFIvsLoNFI.nutrition.P8slope*

Random model:

replicate group + *dam ident*

Correlation between the rate of change of measured blood parameters

Correlations between the rates of change of the different measured parameters were produced for the 2008 post-calving results using the *correlation* function in GenStat 11th edition (VSN International Ltd, Hertfordshire, UK). The correlation coefficients were tested for significance to determine if they were different from zero.

6.5 Results

The main effects of Genotype and nutrition are reported in this section. Means of the main effect are presented in bar charts. Means from both years of the experiment are presented on the same bar chart. Where interactions between the two main effects exist, these means are also presented on bar charts, but each bar chart represents one year of the experiment only.

The associations between covariate measures and blood parameters are presented in tables. For each parameter and covariate effect a P-value to indicate the level of significance of the association between the covariate and the measured parameter is presented. Effects are linear regression coefficients such that an increase of 1 unit of the covariate measure results in a change in the measured parameter equal to the effect.

In this section animals described as “fatter” were identified as having significantly larger US measures of P8 fat than those to which they were compared in the analysis. Conversely “leaner” animals were identified as having significantly smaller US measures of P8 fat than those to which they were compared in the analysis. “Taller” animals were identified as those that had significantly larger height measures.

6.5.1 Pre-calving blood parameter analysis

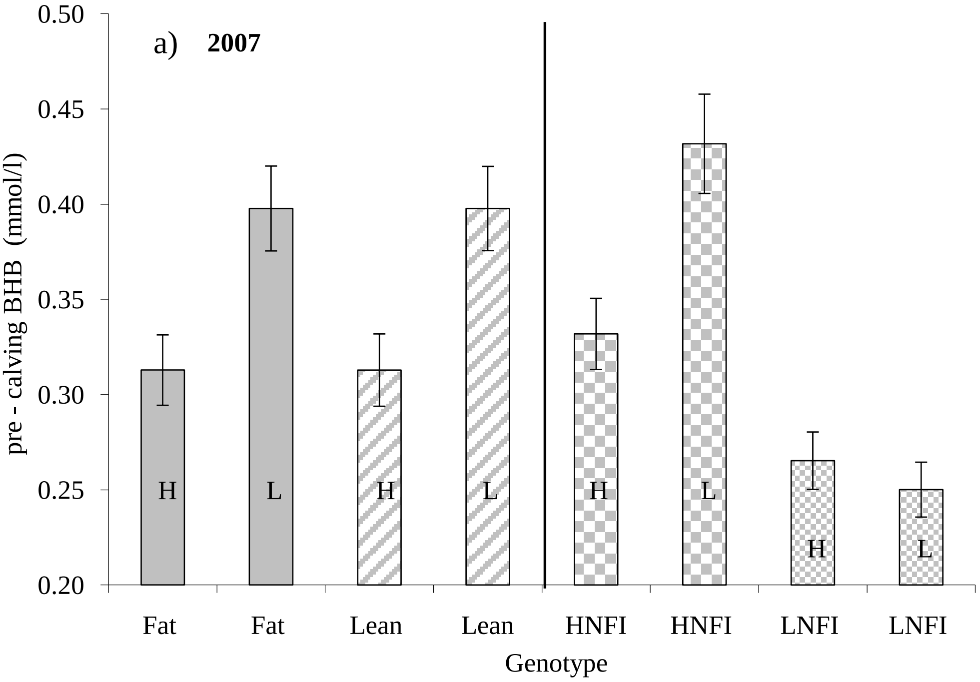
Beta-hydroxybutyrate

In 2007 there were significant interactions between nutrition and FatvsLean ($P = 0.056$) and between nutrition and HiNFIvsLoNFI ($P = 0.027$). Animals on high-nutrition had lower BHB concentrations than animals on low-nutrition in the Fat, Lean and high-NFI Genotypes ($P < 0.05$); there was no significant difference between nutritional treatments on the low-NFI Genotype. High-NFI animals on the low-nutrition treatment had higher BHB concentrations than low-NFI animals on the low-nutrition treatment (see Figure 6-1:a).

In 2008, this effect, of lower BHB concentrations in the high-nutrition treatment, was there in Fat, high-NFI and low-NFI animals ($P < 0.005$) but not in the lean animals (see Figure 6-1:b). Lean animals on high-nutrition had higher BHB than Fat animals on high-nutrition.

Table 6-1 shows the significance level (P-values) and the effect of height, calving date and 3rd Trimester P8 on pre-calving blood parameters in 2007 and 2008. Height was significantly associated with BHB concentrations in 2007 ($P = 0.002$) but not in 2008. Fatter animals (animals with higher US measures of P8 fat) had higher BHB concentrations than leaner animals (2007 $P = 0.008$; 2008 $P < 0.001$). Animals that had blood samples collected from them closer to their calving date had higher BHB concentrations than those sampled further away from their calving date ($P < 0.001$). In 2008 the 1st cohort had higher circulating BHB concentrations than the 2nd cohort ($P < 0.001$).

Significance values (P-values) and LMM model structure used for the analysis of pre-calving BHB concentrations are shown in Table 9-8 and Table 9-9.



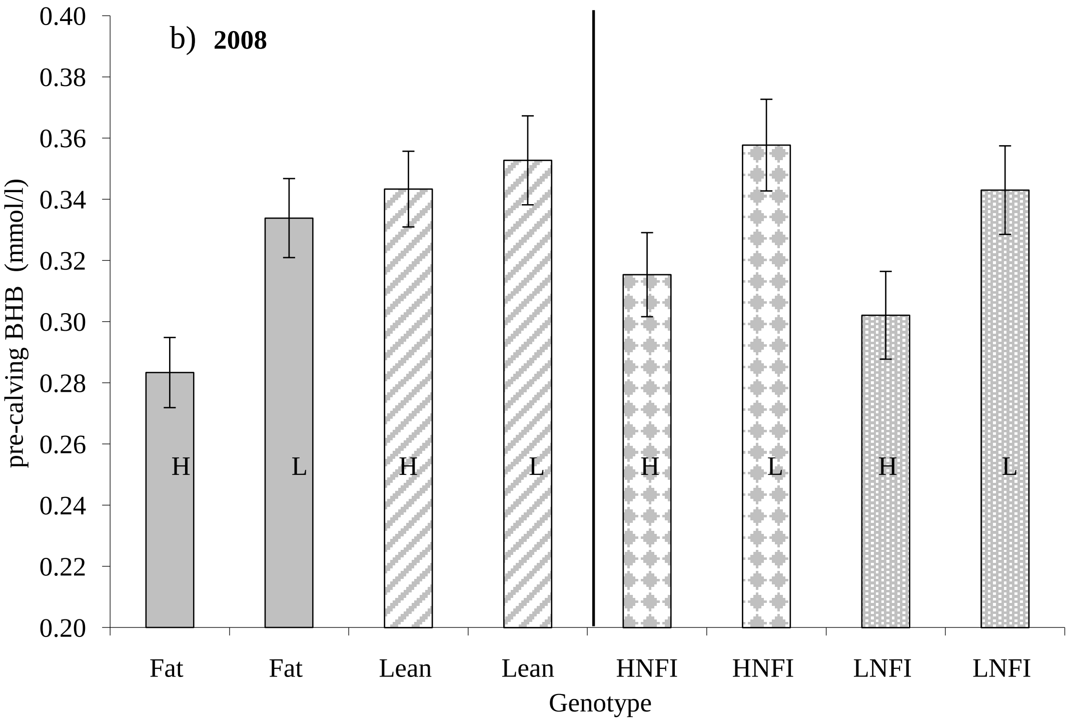


Figure 6-1: Interaction between the effect of Genotype and nutrition on pre-calving BHB (mmol/l) in a) 2007 and b) 2008. High-nutrition (H), low-nutrition (L). Error bars represent 68% confidence intervals.

Table 6-1: Significance level (P-value) and effect of covariates (height, calving date and 3rd trimester P8) on pre-calving blood parameters in 2007 and 2008.

	2007					
	height Aug 07 (cm)		calving date (day)		3rd trimester P8 (mm)	
	Effect	P-value	Effect	P-value	Effect	P-value
pre-calving BHB	0.9987	**0.002**	1.0002	0.851	1.0114	**0.008**
pre-calving glucose	0.0014	0.785	0.0006	0.779	-0.0008	0.591
pre-calving acetate	0.0018	0.510	-0.0007	**0.058**	0.0008	0.810
pre-calving NEFA	0.9986	0.104	0.9949	**<0.001**	1.0289	**<0.001**
pre-calving leptin	-0.0124	**0.025**	0.0017	0.606	0.0752	**<0.001**
pre-calving IGF-1	0.9849	**<0.001**	0.9997	0.573	1.0381	**<0.001**
pre-calving insulin	0.9810	**0.031**	1.0030	0.157	1.0198	0.436
pre-calving GH	0.9868	**0.005**	0.9979	0.256	0.9624	**<0.001**
pre-calving P8	0.0415	**<0.001**	-0.0105	0.445	N/A	N/A
post-calving P8 change	0.0008	**0.032**	-0.0001	0.769	N/A	N/A
	2008					
	height Jan 08 (cm)		calving date (day)		3rd trimester P8 (mm)	
	Effect	P-value	Effect	P-value	Effect	P-value
pre-calving BHB	0.9982	0.261	1.0069	**<0.001**	1.0130	**<0.001**
pre-calving glucose	-0.0011	0.785	-0.0006	0.198	0.0001	0.096
pre-calving acetate	-0.0008	0.183	0.0003	0.150	-0.0013	**0.046**
pre-calving NEFA	1.0071	**0.002**	0.9910	**<0.001**	1.0122	**<0.001**
pre-calving leptin	1.0048	**0.005**	1.0032	**0.001**	1.0083	**<0.001**
pre-calving IGF-1	0.9970	**0.026**	1.0015	0.097	1.0087	**<0.001**
pre-calving insulin	0.9947	**0.041**	0.9986	**0.003**	1.0197	**<0.001**
pre-calving GH	0.9972	0.207	0.9805	**<0.001**	0.9697	**<0.001**
pre-calving P8					N/A	N/A
post-calving P8 change	-0.00053	**0.006**	-0.00015	**0.037**	N/A	N/A

Glucose

In 2008 animals on high-nutrition had higher glucose concentrations than those on low-nutrition ($P < 0.001$, see Figure 6-2).

In 2008 older cows had significantly ($P < 0.001$) lower pre-calving glucose concentrations than younger animals ($P < 0.001$; 1st cohort 3.683 mmol/l ± 0.02075; 2nd cohort 3.855 mmol/l ± 0.0227).

Significance values (P-values) and LMM model structure used for the analysis of pre-calving glucose concentrations are shown in Table 9-8 and Table 9-9.

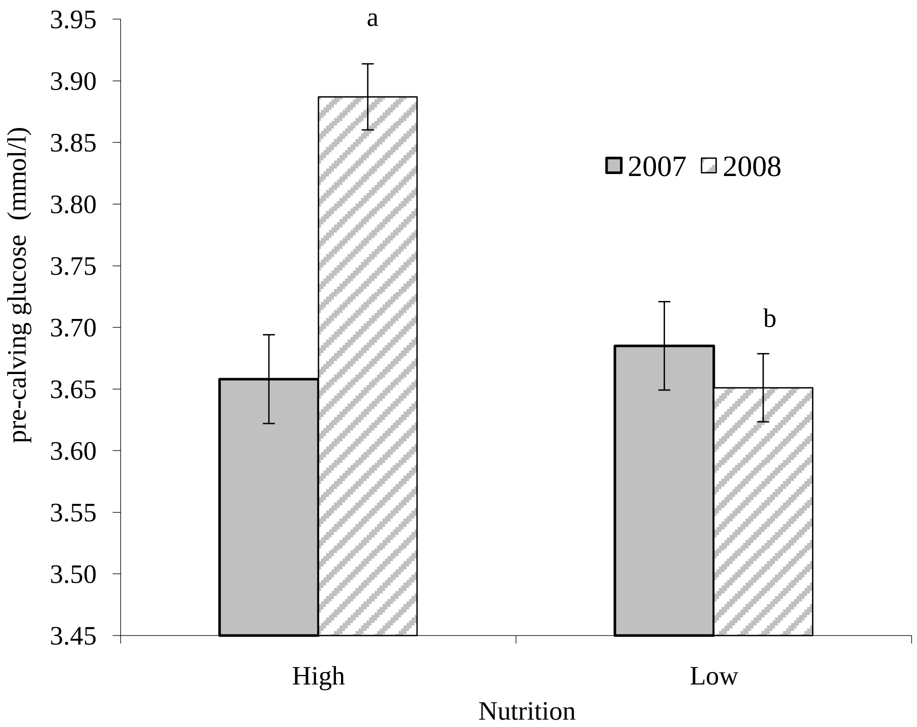


Figure 6-2: Main effect of nutrition on pre-calving glucose concentrations (mmol/l) in 2007 and 2008. Within year, means with different letters differ significantly (P < 0.05). Error bars represent 68% confidence intervals.

Acetate

In 2007 animals on the low-nutrition treatment had higher mean acetate concentrations than animals on high-nutrition (P =0.007, see Figure 6-3). Acetate was associated with 3rd trimester P8 in 2008 but not 2007 (P = 0.046, Table 6-1), and with calving date in 2007 but not in 2008 (P = 0.005). As animals got fatter, and as the date of calving became closer to the end of the calving period, acetate concentrations fell (Table 6-1).

Significance values (P-values) and LMM model structure used for the analysis of pre-calving acetate concentrations are shown in Table 9-8 and Table 9-9.

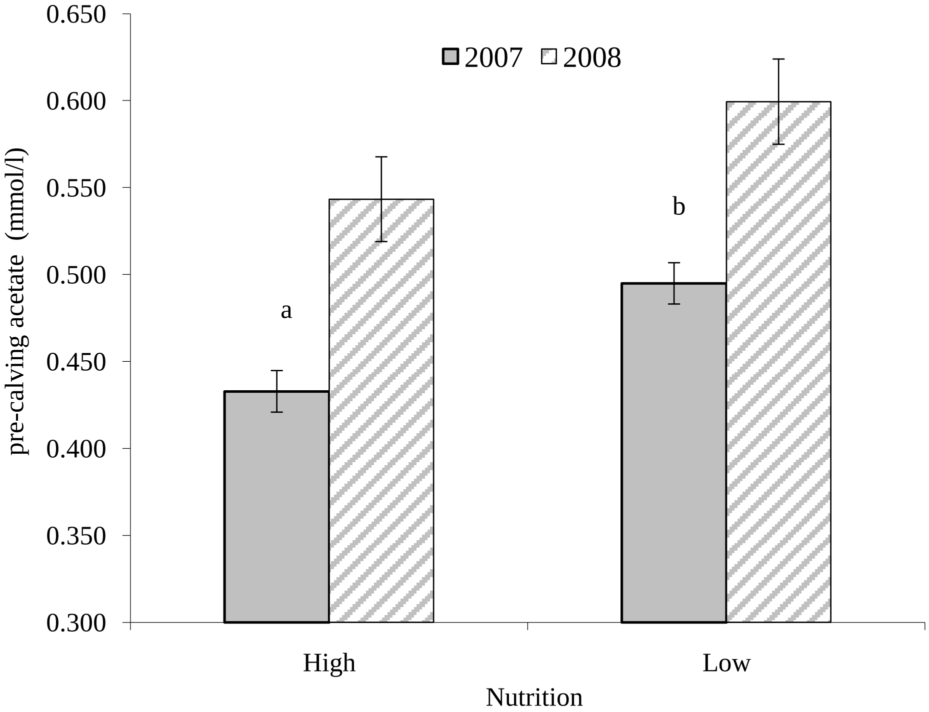


Figure 6-3: Main effect of nutrition on pre-calving acetate concentrations (mmol/l) in 2007 and 2008. Within year, means with different letters differ significantly (P < 0.05). Error bars represent 68% confidence intervals.

Non-esterified fatty acids

Fat animals had higher NEFA concentrations than Lean animals at both levels of nutrition (P =0.026;). On low-nutrition, high-NFI animals had higher NEFA concentrations than low-NFI animals, but on high-nutrition high-NFI animals had lower NEFA concentrations (HiNFIvsLoNFI x Nutrition: P =0.040). Low-nutrition animals had higher NEFA concentrations than high-nutrition animals for all Genotypes except the low-NFI animals (Figure 6-4).

NEFA concentrations were associated with calving date (P < 0.001) and 3rd trimester P8 (P < 0.001) in both 2007 and 2008 (Table 6-1). As animals got fatter, and as date of calving progressed, NEFA concentrations increased. Height (P = 0.002) and

Cohort (P = 0.002) were associated with NEFA concentrations in 2008. Taller or older (1st cohort) animals had higher NEFA concentrations than less tall and younger (2nd cohort) animals.

Significance values (P-values) and LMM model structure used for the analysis of pre-calving NEFA concentrations are shown in Table 9-8 and Table 9-9.

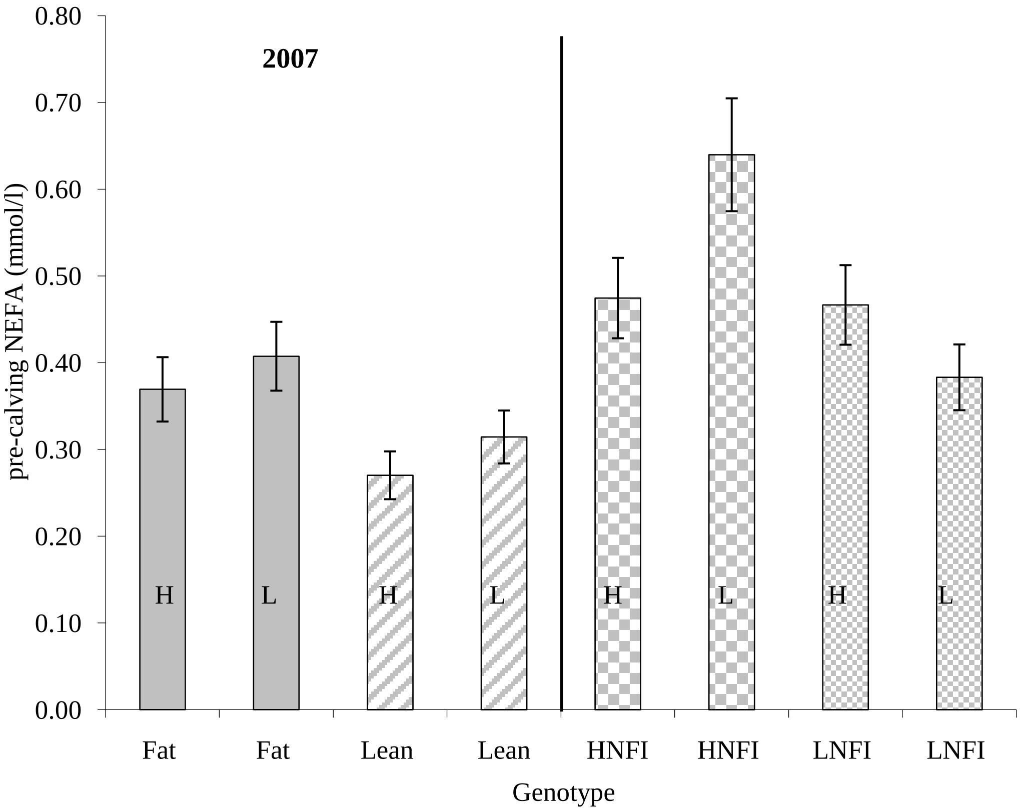


Figure 6-4: Average pre-calving NEFA (mmol/l) for each Genotype and nutritional treatment in 2007. High-nutrition (H), low-nutrition (L). Error bars represent 68% Confidence Intervals.

Leptin

Fat animals had higher circulating leptin concentrations than Lean animals in both 2007 and 2008 ($P < 0.001$, see Figure 6-5), while there was no difference between high- and low-NFI animals. In 2008 animals on high-nutrition had higher leptin concentrations than animals on low-nutrition ($P = 0.026$, see Figure 6-5).

Height ($P < 0.02$) and 3rd trimester P8 fat ($P < 0.001$) were associated with leptin concentrations in 2007 and 2008. Taller and fatter animals had higher leptin concentrations than less tall and leaner animals (Table 6-1). In 2008 older animals (1st cohort) had higher leptin concentrations than younger (2nd cohort) animals ($P < 0.001$). Significance values (P-values) and LMM model structure used for the analysis of pre-calving leptin concentrations are shown in Table 9-8 and Table 9-9.

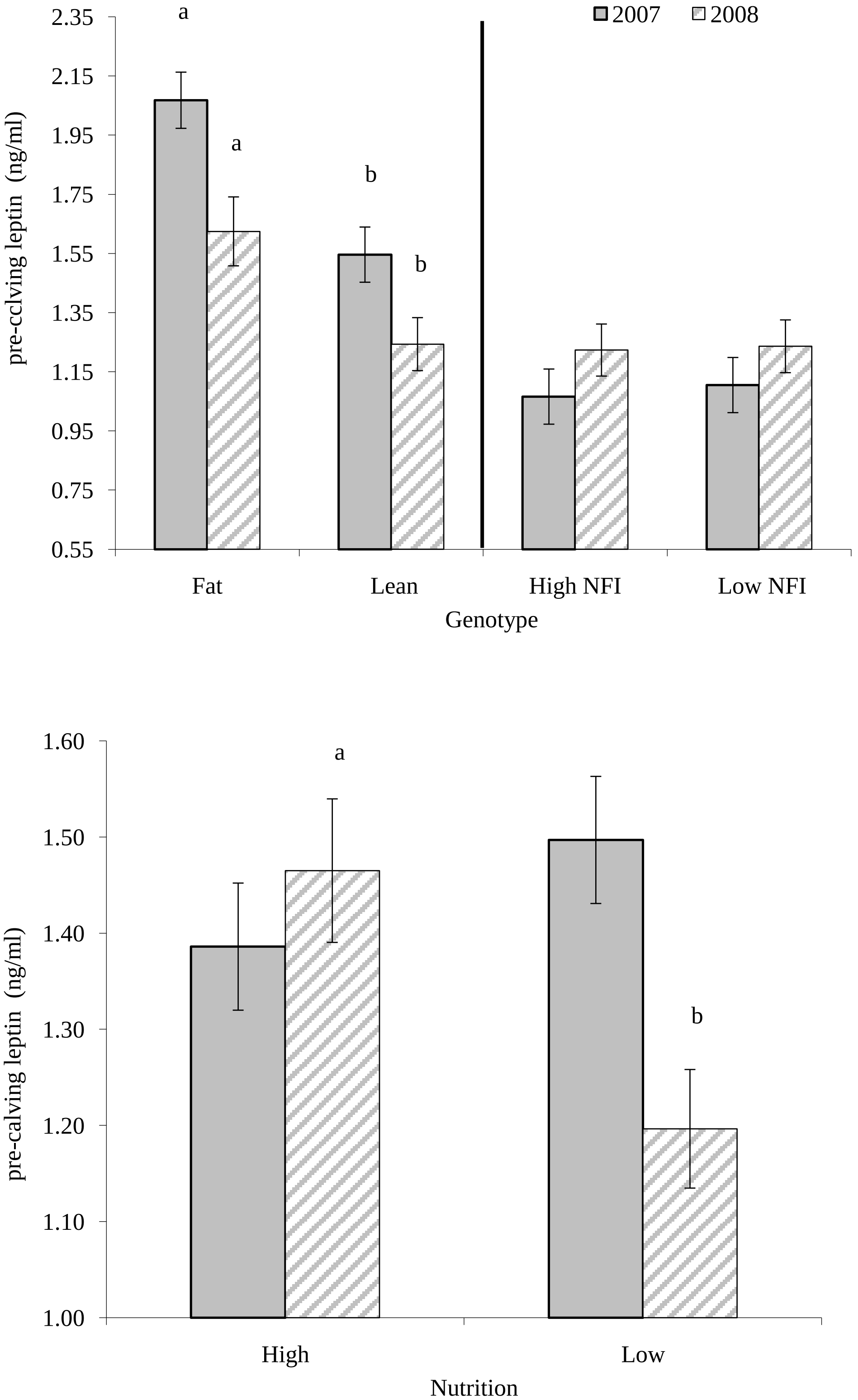
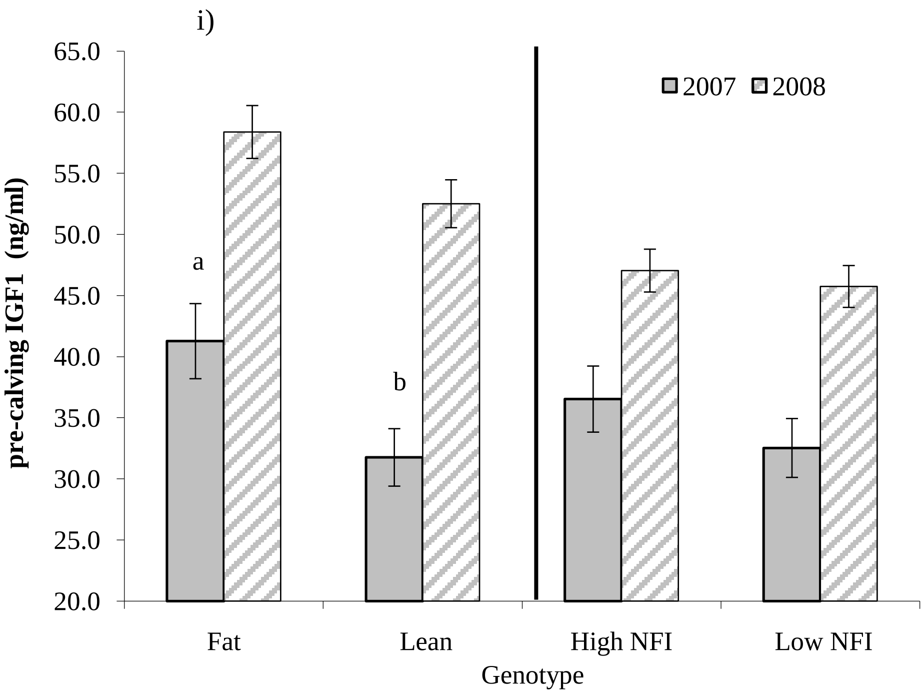


Figure 6-5: Average pre-calving leptin concentrations (ng/ml) for each (i) Genotype and (ii) nutrition treatment in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent 68% Confidence Intervals.

Insulin-like Growth Factor-1

In 2007 Fat animals had significantly higher IGF-1 concentrations than Lean animal (P = 0.032, see Figure 6-6). In 2008 the trend was the same but was not significant (P =0.105). There was no difference in IGF-1 concentrations between high- or low-NFI animals in either year. In 2007 and 2008 animals on high-nutrition had higher IGF-1 concentration than animals on low-nutrition (P = 0.040 and P =0.246, respectively). In both years height and 3rd trimester P8 were associated with IGF-1 concentrations (P =0.001; Table 6-1) whereby taller or fatter animals had higher concentrations than less tall or leaner animals. Cohort was not associated with IGF-1 in 2008.

Significance values (P-values) and LMM model structure used for the analysis of pre-calving IGF-1 concentrations are shown in Table 9-8 and Table 9-9.



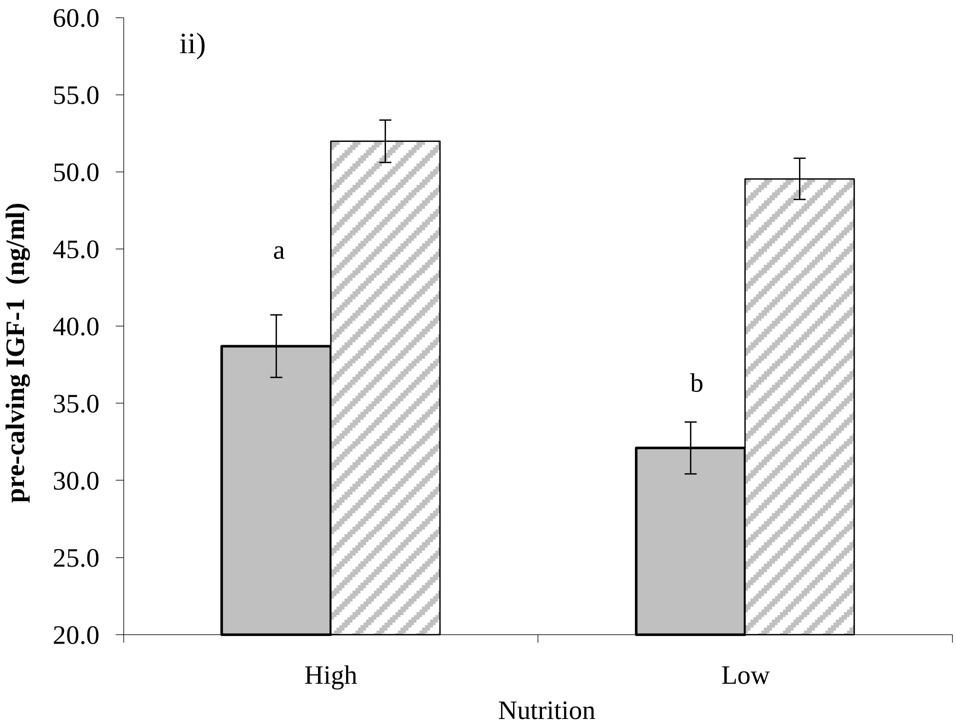


Figure 6-6: Average pre-calving IGF-1 concentrations (ng/ml) for each (i) Genotype and (ii) nutrition treatment in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent 68% Confidence Intervals.

Insulin

There was an interaction between nutrition and the comparison between high- and low-NFI in 2007 (P =0.008, Figure 6-7) . Low-NFI animals on low-nutrition had higher insulin concentrations (µmol/ml) than low-NFI animals on high-nutrition (P<0.05) but there was no effect of nutrition for high-NFI animals. Fat animals had higher insulin concentrations than lean animals and this effect did not change with level of nutrition. In 2008 animals on low-nutrition had lower insulin concentrations than animals on high-nutrition ((P = 0.023) and there were no effect of Genotype.

Taller animals had higher concentrations of insulin than shorter animals (2007 P = 0.031, 2008 P = 0.041, see Table 6-1). Calving date and 3rd trimester P8 were associated with insulin concentrations in 2008 but not in 2007 (P = 0.003; P < 0.001 respectively). As calving date got closer to the end of the calving period and as animals got fatter insulin concentrations increased (Table 6-1).

Significance values (P-values) and LMM model structure used for the analysis of pre-calving insulin concentrations are shown in Table 9-8 and Table 9-9.

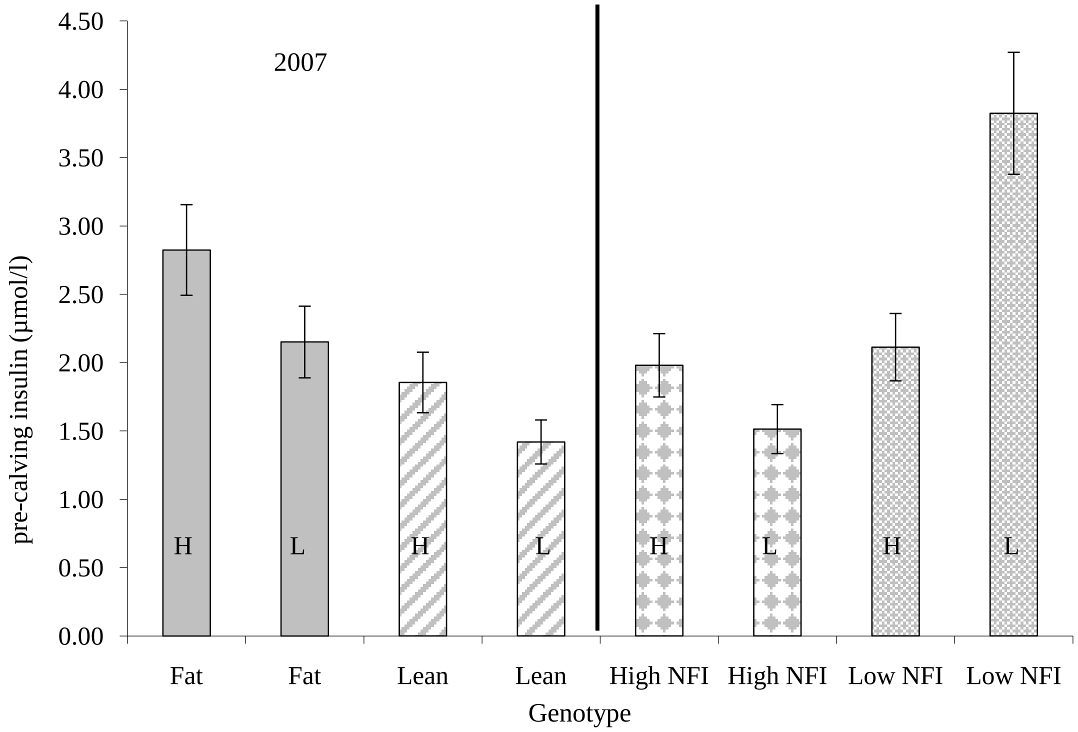


Figure 6-7: Average pre-calving insulin (µmol/l) in 2007 for each Genotype on high-nutrition (H) and low-nutrition (L). Error bars represent 68% Confidence Intervals.

Growth hormone

In 2007 Lean animals and low-NFI animals had higher GH concentrations than Fat or high-NFI animals respectively ($P < 0.001$, see Figure 6-8).

In both years 3rd trimester P8 was associated with mean pre-calving GH whereby GH concentrations increased as P8 fat depth increased. ($P < 0.001$, see Table 6-1). In 2007 taller animals had higher GH concentrations than less tall animals ($P = 0.005$). In 2008 cohort ($P < 0.001$) and calving date ($P < 0.001$) were significantly associated with GH concentrations, with younger animals having higher GH concentrations than older animals, and as calving date came closer to the end of the calving period GH concentrations increased.

Significance values (P-values) and LMM model structure used for the analysis of pre-calving GH concentrations are shown in Table 9-8 and Table 9-9.

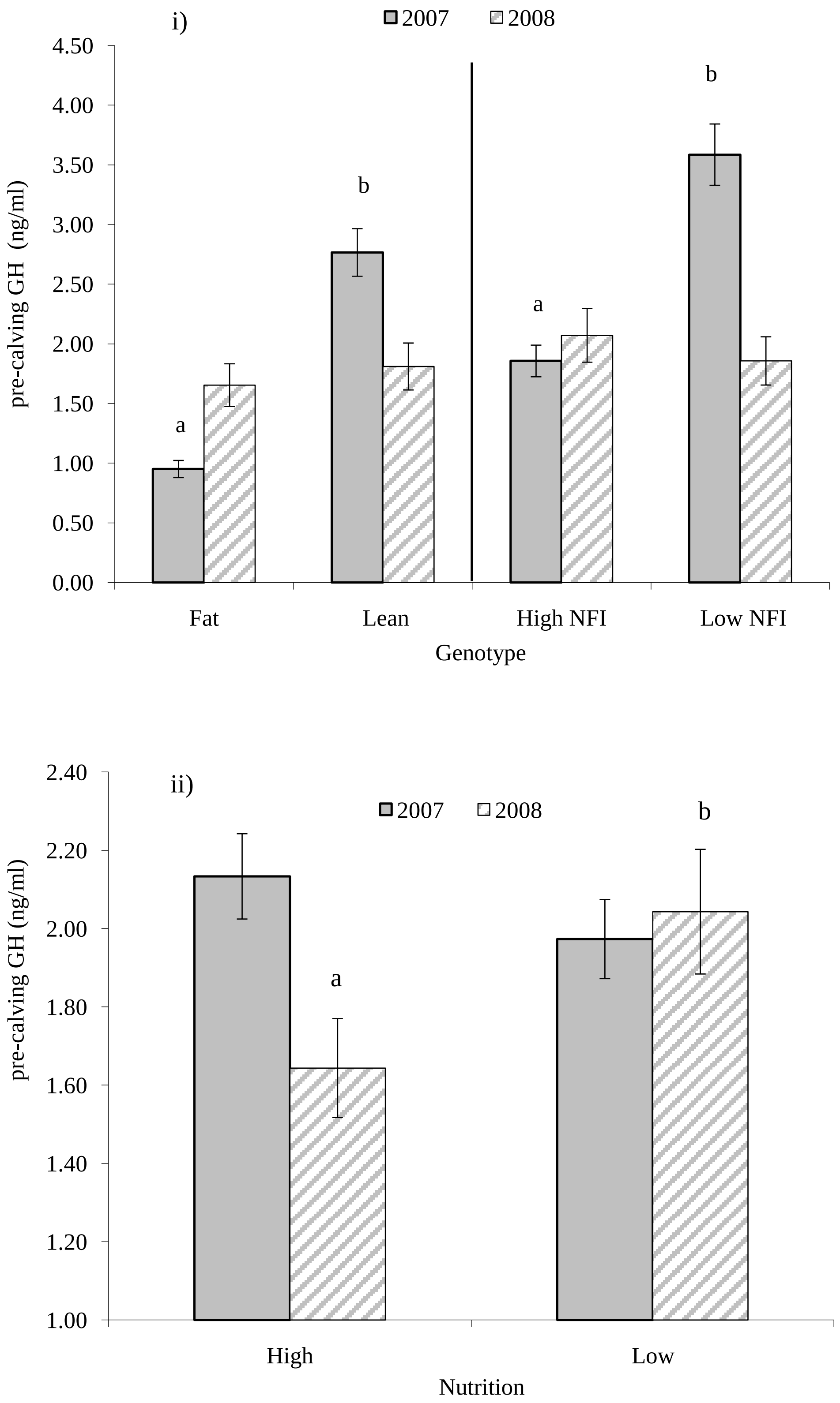


Figure 6-8: Main effects of Genotype (i) and nutrition (ii) on pre-calving GH concentrations (ng/ml) in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent 68% confidence intervals.

6.5.2 *Post-calving blood parameter analysis*

All P–values and effects of days-post-calving on blood parameters in 2007 and 2008 are listed in Table 6-2 and the effects and significance of the covariates on these parameters are listed in Table 6-3. The significance of the main effects of Genotype and nutrition and all interactions are shown in 9.6.

Beta-hydroxybutyrate

In 2007 and 2008 lactating animals had significantly higher mean circulating BHB concentrations than non-lactating animals ($P = 0.019$ 2007; $P < 0.001$ 2008, see Figure 6-9). In 2008 taller animals had higher BHB concentrations ($P = 0.050$). Younger animals (2^{nd} cohort) had higher mean post-calving BHB concentrations than older animals (1^{st} cohort, $P = 0.014$).

In 2008 samples collected later in the post-calving period had higher BHB concentrations than early samples (see Table 6-2). This indicates there is a significant linear increase in BHB from calving until the end of the sampling period. This relationship is explained in more detail through interactions between the main effects of Genotype and nutrition and days-post-calving.

BHB in Fat animals increased faster than in Lean animals during the post-calving period ($P = 0.002$). BHB increased faster on the low-nutrition treatment compared to the high-nutrition treatment ($P < 0.001$, see Table 6-4).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving BHB concentrations are shown in Table 9-10 and Table 9-11.

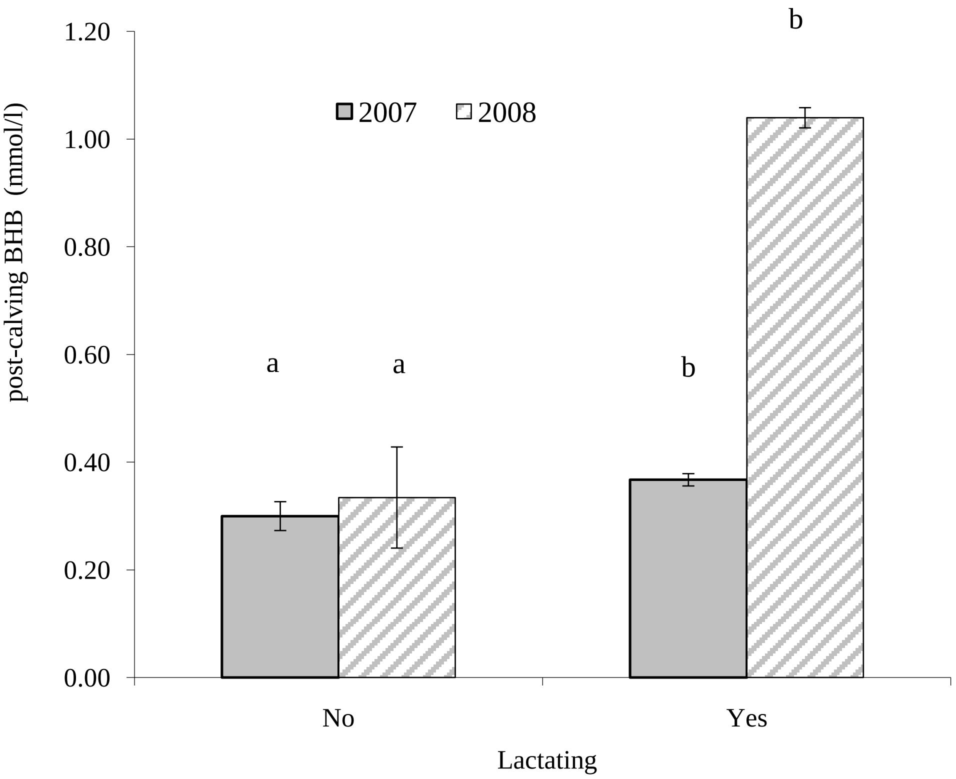


Figure 6-9: Main effect of lactation on post-calving BHB concentrations (mmol/l) in 2007 and 2008. Means with different letters differ significantly (P < 0.05). Error bars represent 68% confidence intervals.

Table 6-2: Effect (unit change/day) and level of significance (P-value) of days-post-calving on post-calving blood parameters in 2007 and 2008.

	days-post-calving		days-post-calving	
	2007		2008	
	Effect	P-value	Effect	P-value
post-calving BHB	0.0010	0.334	0.0020	**<0.001**
post-calving glucose	-0.0030	**0.001**	-0.0060	**<0.001**
post-calving acetate	-0.0006	0.209	0.0014	**<0.001**
post-calving NEFA	0.0080	**<0.001**	N/A	N/A
post-calving leptin	-0.0020	0.171	0.0044	**<0.001**
post-calving IGF-1	-0.0030	0.093	0.0010	**<0.001**
post-calving insulin	0.0000	0.761	0.0000	0.203
post-calving GH	0.0004	0.892	-0.0025	**<0.001**

Table 6-3: Effect (unit change/day) and level of significance (P-Value) of height, calving date and 3rd trimester P8 on post-calving blood parameters in 2007 and 2008

	2007							
	height Aug 07		calving date		pre-calving measure		3rd trimester P8	
	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value
post-calving BHB	0.993	0.546	1.001	0.518	1.123	0.225	1.005	0.717
post-calving glucose	0.002	0.887	-0.001	0.570	0.122	0.297	-0.001	0.875
post-calving acetate	-0.001	0.423	0.000	0.818	0.459	**0.002**	0.006	0.929
post-calving NEFA	0.992	0.115	1.015	**0.001**	1.204	0.189	0.990	0.954
post-calving leptin	0.012	0.349	-0.003	0.700	0.944	**<0.001**	-0.003	**<0.001**
post-calving IGF-1	1.008	0.338	0.991	**<0.001**	1.477	**0.002**	0.992	0.834
post-calving insulin	1.005	0.513	0.997	0.394	1.118	**0.045**	1.006	0.843
post-calving GH	1.000	0.576	0.998	0.219	1.281	**<0.001**	0.989	**0.066**
	2008							
	height Aug 07		calving date		pre-calving measure		3rd trimester P8	
	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value
post-calving BHB	1.004	**0.050**	1.002	0.431	1.110	**0.022**	1.003	0.115
post-calving glucose	0.001	0.479	-0.002	**0.015**	0.199	**<0.001**	0.001	0.102
post-calving acetate	0.001	0.384	0.001	0.543	0.270	**<0.001**	0.000	0.282
post-calving leptin	0.999	0.142	0.998	**0.001**	2.484	**<0.001**	1.004	**<0.001**
post-calving IGF-1	1.007	0.127	0.997	**0.008**	1.789	**<0.001**	0.998	0.205
post-calving insulin	1.001	0.806	1.000	0.548	1.102	0.006	1.003	**0.002**
post-calving GH	1.003	0.549	1.001	**0.015**	1.276	**<0.001**	0.990	**<0.001**

Table 6-4: Effects of days-post-calving for each Genotype and nutrition in 2007 and 2008 and their significance (P-value). Only significant interactions are displayed

	2007 Interactions: days-post-calving								
	Effect		P-value	Effect		P-value	Effect		P-value
	Fat	Lean		high-NFI	low-NFI		high-nutrition	low-nutrition	
post-calving BHB	-	-	-	-	-	-	-	-	-
post-calving glucose	-	-	-	-	-	-	-	-	-
post-calving acetate	-	-	-	-	-	-	-	-	-
post-calving NEFA	0.001	0.015	**0.002**	-	-	-	0.003	0.013	**0.011**
post-calving leptin	-	-	-	-	-	-	0.000	-0.004	**0.045**
post-calving IGF-1	-	-	-	-	-	-	-	-	-
post-calving insulin	-	-	-	-	-	-	-	-	-
post-calving GH	-	-	-	-	-	-	-	-	-

	2008 Interactions: days-post-calving								
	Effect		P-value	Effect		P-value	Effect		P-value
	Fat	Lean		high-NFI	low-NFI		high-nutrition	low-nutrition	
post-calving BHB	0.003	0.002	**0.002**	-	-	-	0.002	0.004	**<0.001**
post-calving glucose	-	-	-	-	-	-	-	-	-
post-calving acetate	0.002	0.001	**<0.001**	-	-	-	0.001	0.002	**0.043**
post-calving leptin	-	-	-	-	-	-	-	-	-
post-calving IGF-1	-	-	-	-	-	-	-	-	-
post-calving insulin	-	-	-	-	-	-	-0.002	0.002	**<0.001**
post-calving GH	-	-	**0.005**	-	-	-	-	-	-

Glucose

In 2008 animals on low-nutrition had lower glucose concentrations than those on high-nutrition (P = 0.036, see Figure 6-10). In both years animals sampled later in the post-calving period had significantly lower glucose concentrations than earlier sampled animals (P = 0.001 2007; P < 0.001 2008, see Table 6-2). In 2008 later-calving animals had lower glucose than early-calving animals (P = 0.015, see Table 6-3).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving glucose concentrations are shown in Table 9-10 and Table 9-11.

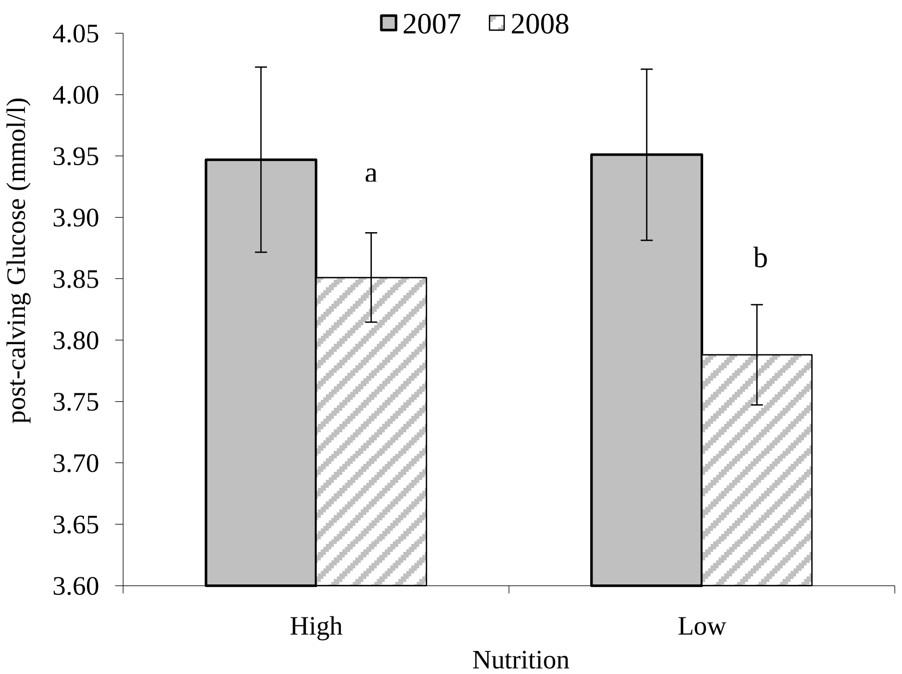


Figure 6-10: Effect of nutrition on post-calving glucose concentrations in 2007 and 2008. Within year means with different letters differ significantly (P < 0.05). Error bars represent 68% confidence intervals.

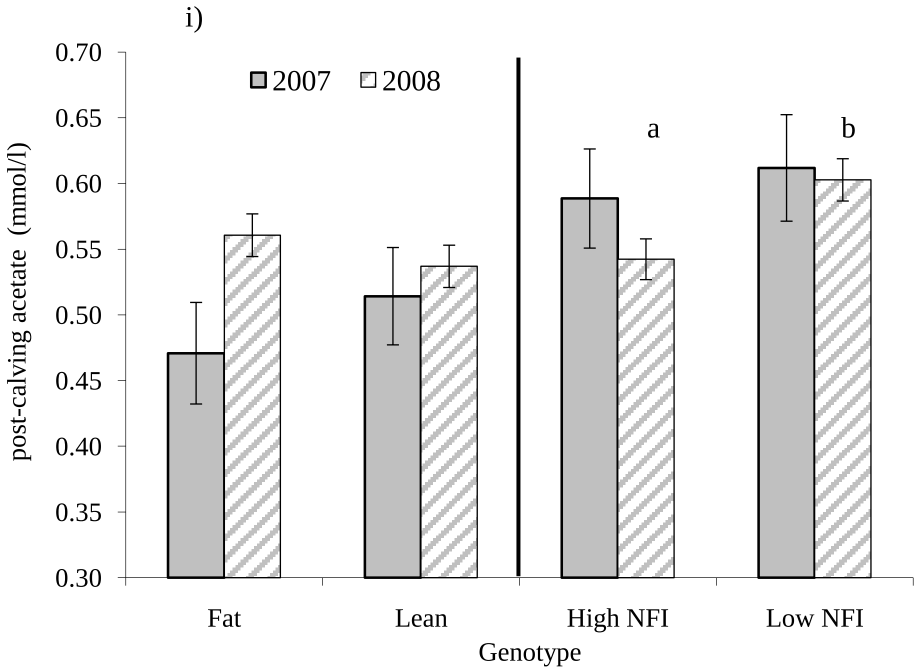
Acetate

In 2008 low-NFI animals had higher circulating acetate concentrations than high-NFI animals (P = 0.020, see Figure 6-11). In both years lactating animals had significantly higher circulating acetate concentrations than non-lactating animals (P = 0.004 2007; P = <0.001 2008; see Figure 6-11).

In 2008 samples collected later had higher acetate concentrations than early samples (see Table 6-2). This indicates there is a significant linear increase in acetate from calving until the end of the sampling period. This relationship is explained in more detail through interactions between the main effects of Genotype and nutrition and days-post-calving.

Acetate in Fat animals increased faster than in Lean animals post-calving ($P < 0.001$). Furthermore, acetate increased faster on the low-nutrition treatment compared to the high-nutrition treatment ($P < 0.001$, see Table 6-4).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving acetate concentrations are shown in Table 9-10 and Table 9-11.



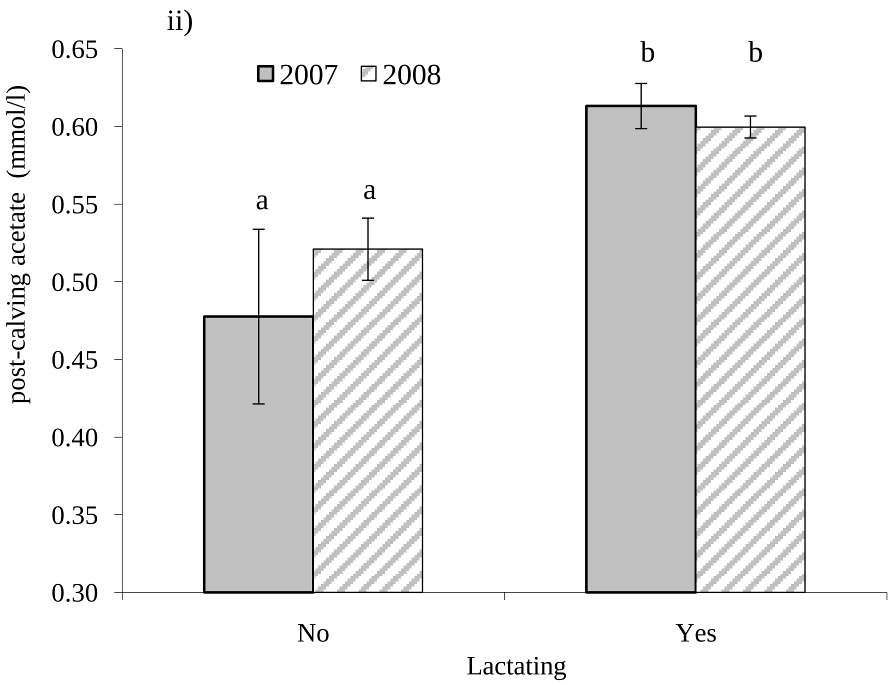


Figure 6-11: Main effects of Genotype (i) and lactation (ii) on post-calving acetate concentrations (mmol/L) in 2007 and 2008. Within line and year, means with different letters differ significantly ($P < 0.05$). Error bars represent 68% confidence intervals.

Non-esterified fatty acids

NEFA was not measured in 2008. In 2007 lactating animals had higher NEFA concentration than non-lactating animals (P = 0.017, see Figure 6-12). NEFA concentrations increased as the calving date became closer to the end of the sampling period.

In 2007 samples collected later after calving had higher NEFA concentrations than early samples (P = <0.001; see Table 6-2). This indicates that there is a significant linear increase in NEFA from calving until the end of the sampling period. This relationship is explained in more detail through the significant interaction between Genotype, nutrition and days-post-calving.

NEFA in Lean animals increased faster than in Fat animals post-calving, and increased faster on the low-nutrition treatment compared to the high-nutrition treatment (P < 0.05, see Table 6-4).

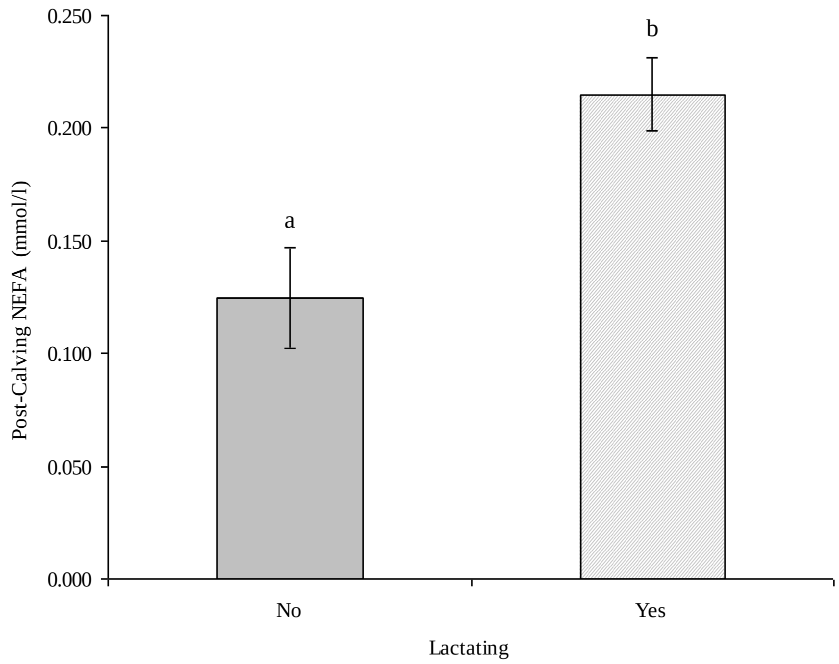


Figure 6-12: Effect of lactation on mean NEFA concentrations (mmol/L) post-calving in 2008. Means with different letters differ significantly (P<0.005). Error bars represent 68% confidence intervals.

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving NEFA concentrations are shown in Table 9-10 and Table 9-11.

Leptin

In 2008 animals on high-nutrition animals had higher circulating leptin concentrations than animals on low-nutrition ($P < 0.001$, see Figure 6-13). In both years fatter animals (higher US measures of P8 fat) had significantly higher leptin concentrations than leaner animals ($P < 0.001$). In 2008 leptin concentrations increased as calving date became later ($P = 0.001$, see Table 6-2). Lactating animals had lower mean leptin concentrations than non-lactating animals (see Figure 6-14).

In 2008 samples collected later had higher leptin concentrations than early samples ($P < 0.001$, see Table 6-2). This indicates that there is a significant linear increase in leptin from calving until the end of the sampling period. This relationship is explained in more detail through a significant interaction between nutrition and days-post-calving

In 2007 leptin increased in animals on high- compared to those on low-nutritional treatments, for which leptin concentrations fell as the number of days-post-calving increased (see Table 6-4).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving leptin concentrations are shown in Table 9-10 and Table 9-11.

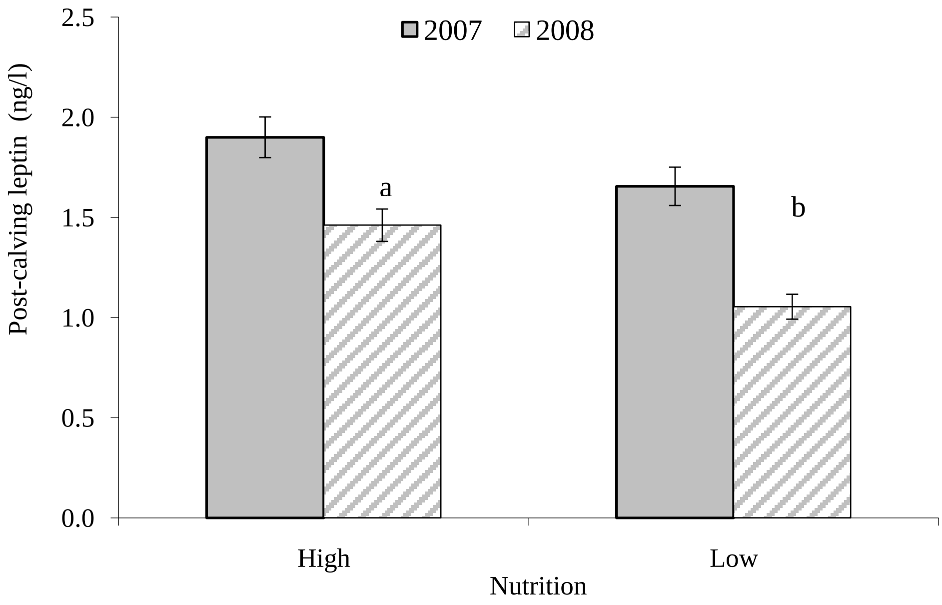


Figure 6-13: Effect of nutrition on circulating leptin concentrations (ng/ml) in 2007 and 2008. Means with different letters differ significantly (P<0.005). Error bars represent 68% confidence intervals.

Figure 6-14: Effect of lactation on mean leptin concentrations (ng/ml) post-calving in 2008. * indicates means differ significantly (P<0.005). Error bars represent 68% confidence intervals.

Insulin-like Growth Factor-1

In 2007 IGF-1 concentrations increased as calving date became later ($P<0.001$). In 2008 lactating animals had significantly lower IGF-1 concentrations than non-lactating animals ($P < 0.001$, see Figure 6-15) and as sampling date ($P < 0.001$) and calving date ($P = 0.008$) became later IGF-1 concentrations increased (see Table 6-2). Younger animals (2[nd] cohort) had lower IGF-1 concentrations than older animals (1[st] cohort) (results not displayed, $P < 0.001$).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving IGF-1 concentrations are shown in Table 9-10 and Table 9-11.

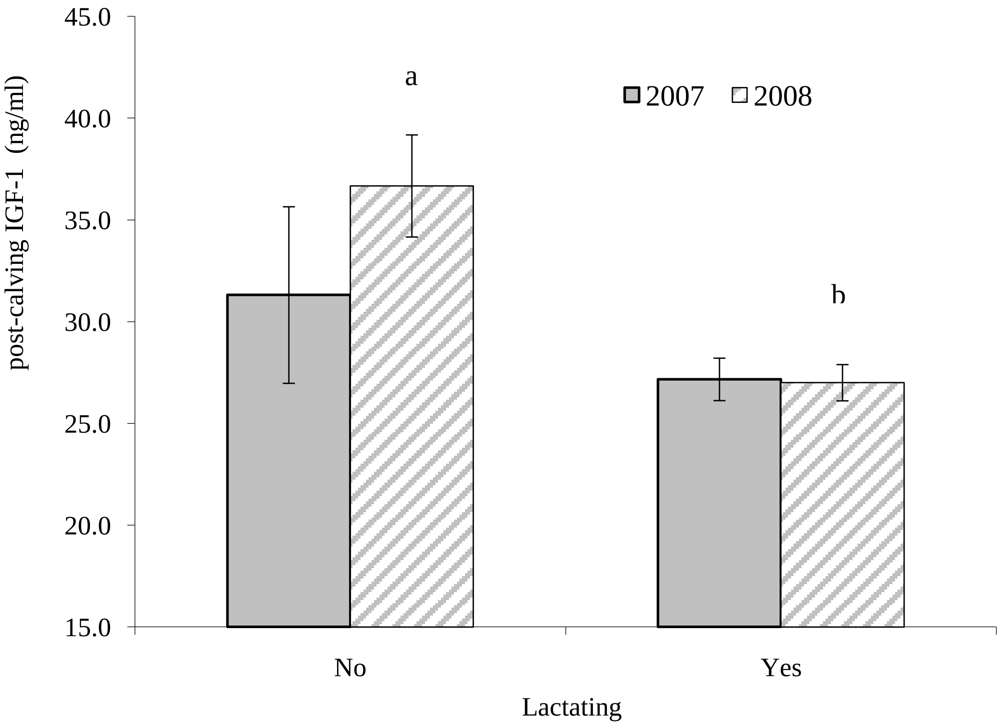


Figure 6-15: Effect of lactation on circulating IGF-1 concentrations post-calving in 2007 and 2008. Means with different letters differ significantly ($P<0.005$). Error bars represent 68% confidence intervals.

Insulin

In 2008 3rd trimester P8 was associated with mean post-calving insulin where animals with higher measures of 3rd trimester P8 fat higher circulating insulin concentrations than leaner animals (P = 0.002, see Table 6-3).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving insulin concentrations are shown in Table 9-10 and Table 9-11.

Growth Hormone

In 2008 animals on low-nutrition had higher circulating GH concentrations than animals on high-nutrition (P < 0.001, See Figure 6-16).

In 2008 samples collected later had lower GH concentrations than early samples (P < 0.001 see Table 6-2). This indicates that there is a significant linear decrease in GH from calving until the end of the sampling period. This relationship is explained in more detail through the significant interaction between Genotype and days-post-calving.

As calving came closer to the end of the calving period in 2008, GH increased significantly (P = 0.015, see Table 6-2). In both years fatter animals had higher mean GH concentrations than leaner animals (P< 0.001, see Table 6-3).

Significance values (P-values) and LMM model structure used for the analysis of mean post-calving GH concentrations are shown in Table 9-10 and Table 9-11.

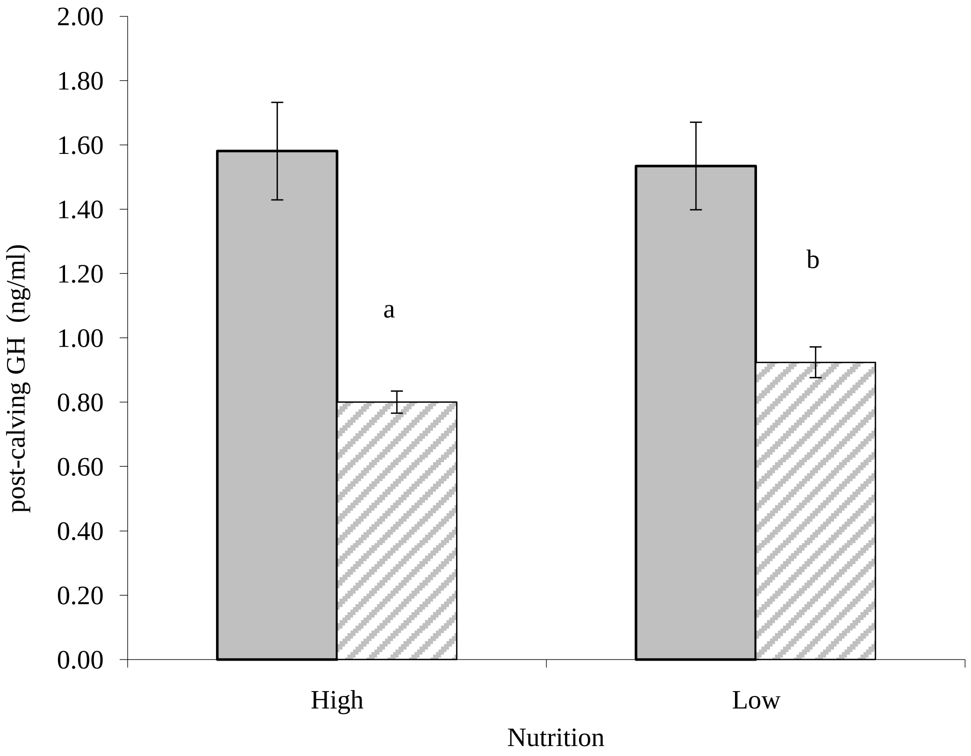


Figure 6-16: Main effect of nutrition on post-calving GH concentrations (ng/ml) in 2007 and 2008. Means with different letters differ significantly (P<0.005). Error bars represent 68% confidence intervals.

Correlation between blood parameters

To examine the relationship between the mean post-calving concentrations of blood parameters in 2008 an analysis was done to produce correlations and their significance values (Table 6-5). This table shows a significant correlation between BHB and acetate; leptin and BHB; glucose and acetate and BHB; insulin and leptin, glucose and GH; IGF-1 and BHB and insulin; and the rate of change in P8 fatness and BHB and leptin.

Table 6-5: Correlations and the level of significance (below, P-values) between measured parameters, post-calving in 2008. *** = <0.001, ** = P <0.05, * = P<0.01.

	acetate	BHB	leptin	glucose	GH	insulin	IGF-1	change P8
BHB	0.46 ***							
leptin	0.03 0.632	-0.23 **						
glucose	-0.35 ***	-0.20 *	0.09 0.213					
GH	-0.05 0.488	-0.05 0.454	-0.14 0.061	0.11 0.116				
insulin	-0.08 0.279	-0.11 0.131	0.27 ***	0.42 ***	-0.23 **			
IGF-1	-0.11 0.131	-0.31 ***	0.13 0.068	0.11 0.143	-0.07 0.314	0.16 *		
change P8	0.03 0.656	-0.31 ***	0.18 *	0.03 0.710	0.06 0.426	0.09 0.200	0.10 0.159	

Rate of change of blood parameter compared to changing fatness

An analysis was done to examine the relationship between the post-calving rate of change of each measured parameter and the rate of change of P8 fatness over the calving period.

The rate of change of fatness (P8 change) was associated with the rate of change of leptin post-calving, whereby animals that became fatter more quickly had a significantly faster rate of increase of leptin concentrations post-calving (P = 0.033 see Table 9-12). No other associations were significant.

Significance values (P-values) and LMM model structure used for the analysis of the rate of change of blood parameters compared to changing fatness are shown in Table 9-12.

Correlation between the rate of change of measured blood parameters

There were some significant correlations between the rates of change of several measured blood parameters. These relationships are shown in Table 6-6. There were significant correlations between the rates of change post-calving of BHB and acetate; glucose and acetate and BHB; GH and acetate, BHB and glucose; insulin and glucose; IGF-1 and BHB; and the change in P8 and acetate, BHB and leptin.

Table 6-6: Correlations and the level of significance (below, P-values) of rates of change between measured parameters, post-calving in 2008. *** = <0.001, ** = P <0.05, * = P<0.01.

	change acetate	**change BHB**	**change leptin**	**change glucose**	**change GH**	**change insulin**	**change IGF-1**	**change P8**
change BHB	0.36 ***							
change leptin	-0.02 0.827	0.03 0.710						
change glucose	-0.39 ***	-0.31 ***	-0.05 0.523					
change GH	-0.26 ***	-0.25 ***	-0.03 0.706	0.27 ***				
change insulin	0.01 0.854	-0.12 0.092	0.12 0.093	0.24 ***	-0.05 0.473			
change IGF-1	0.04 0.627	-0.19 **	0.08 0.253	0.11 0.125	0.06 0.418	0.06 0.452		
change P8	-0.15 *	0.18 *	0.16 *	-0.02 0.791	-0.04 0.580	-0.04 0.632	-0.05 0.480	

6.6 Discussion

It has previously been hypothesised that feed intake, diet composition, energy balance, and physical activity of an animal impact more than Genotype does on circulating concentrations of blood metabolites (Beeby *et al.*, 1988; Spicer *et al.*, 2002) and it is often difficult to form conclusions about cause and effect. The following discussion is an attempt to explain the results from the present experiment in the context of the existing literature and to unravel the impact of experimental Genotype on blood parameters pre-and post-calving. It is important to note that the results from Chapter 5 confirm that the nutritional treatment imposed on the animals in the experimental design did lead to a reduced supply of feed, and fewer MJ ME were consumed by the cows on the low-nutrition treatment than on the high-nutrition treatment. It is therefore accepted in this discussion that during 2008 there was a significant difference in energy supply between the two treatments. The differences in the treatments in 2007 are less clear cut as no intake analysis was done; however, results from Chapter 5 suggest that during the post-calving period there was a difference in energy supply, albeit subject to the influence of a changeable growing season, as described in 2.12.1.

It was hypothesised that nutrition, rather than Genotype, would affect blood parameter concentrations, and differences between Genotypes could be explained by differences in energy balance and/or adiposity of the animal. This hypothesis was supported by the results of this experiment, which show that nutritional treatment and fatness significantly affected blood parameter concentrations in animals of differing Genotypes. The following section is a discussion of the specific parameters and how they support this hypothesis.

BHB was more reflective of fatness and nutritional status than other blood parameters were. Genotype differences in BHB concentrations can be explained by differences in adiposity. BHB is the product of fat mobilisation and ketogenesis during a response to negative energy balance (Lucy *et al.*, 1991). Concentrations of BHB were negatively correlated to IGF-1 (r = -0.31) in this experiment, and IGF-1 is an indicator of energy balance in ruminants. Genotype and nutrition interacted in both years of the experiment and the results support the hypothesis that energy balance and fatness rather than Genotype per se influenced blood parameters.

The difference in fatness between Fat vs. Lean, and high-NFI vs. low-NFI animals has already been reported in Chapter 3 (see Figure 3-2 and Figure 3-5). The differences in BHB concentrations between the Genotypes, both pre- and post-calving, tended to exist in a similar pattern to the differences in fatness. It was hypothesised that the Genotype differences in BHB concentrations were influenced directly by adiposity. High-NFI and Fat animals were fatter and had higher BHB concentrations than low-NFI and Lean animals, a result similar to that reported by Kelly *et al.* (2010). It was postulated that fatter animals had more fat to mobilise in times of energy demand and as a consequence BHB concentrations were higher in those animals. The significant Genotype by nutrition interaction pre-calving in 2007, whereby fatter animals (Fat and high-NFI) on low-nutrition had higher serum BHB than fatter animals on high-nutrition, suggests that under greater nutritional pressure, fatter animals produced more BHB when the demand was present. When extra production of alternative energy sources such as BHB was not required, such as when nutrition was adequate, BHB concentrations were not elevated. The absence of the nutrition effect on a plane of good nutrition indicates that when energy supply was adequate the demand for alternative energy sources such as ketones was minimal.

Energy demand impacted on mean post-calving BHB concentrations. Although the mean difference in BHB concentrations between the nutritional treatments post-calving was not significantly different in 2008, the rate of increase during the post-calving period was higher for the low-nutrition treatment. This suggests that the animals on low-nutrition were in a state of greater energy deficit and the need to mobilise fat to produce energy alternatives was higher than it was when nutrition was adequate. In addition, lactation had a very significant effect. Lactating animals had significantly higher BHB in both years post-calving which was expected because it was these animals that were subjected to the massive energy burden of milk production. Ketogenesis would be a significant contributor to the energy supply in lactating animals but insignificant in non-lactating cows. The conclusion that mean post-calving BHB concentrations reflected the energy balance of the cow in the current experiment is consistent with the findings in other literature (Ingvartsen and Andersen, 2000).

It has been shown that primiparous cattle had lower BCS and produced less milk post partum than multiparous animals (Meikle *et al.*, 2004) and those researchers also noted that the BHB concentrations of primiparous post-calving were on average higher than in multiparous animals. This concurs with results from the current experiment which showed that cohort affected ($P < 0.001$) pre-calving BHB concentrations in that the animals in the 2nd cohort (primiparous) had higher circulating BHB concentrations than the 1st cohort (multiparous) of animals (1st cohort 0.360 mmol/l ± 0.0269; 2nd cohort 0.363 mmol/l ± 0.0275). This result was expected because younger animals were shown to be significantly ($P < 0.001$) leaner pre-calving (see Section 3.5.2) and therefore energy reserves were lower and the demand for ketogenesis to meet energy deficits was higher. Compounding this effect was that the young animals still had an energy requirement for growth as well as having a lower feed capacity (Remond *et al.*, 1991). Supporting this hypothesis is the fact that IGF-1 concentrations post-calving were also significantly affected by cohort in that younger animals had lower serum IGF-1 than multiparous animals ($P < 0.001$). This is a clear indication that the energy deficit was greater in the primiparous animals and hence the higher serum BHB post-calving.

The experimental results showed that serum leptin was also closely associated with body fatness. Leptin is synthesised by white adipocytes (Chilliard *et al.*, 2001; Macajova *et al.*, 2004) and has a role in the regulation of appetite (Houseknecht *et al.*, 1998), reproductive performance (Hileman *et al.*, 2000) and food intake (Howie, 1999), and it also affects body composition (Fitzsimmons *et al.*, 1998; Schenkel *et al.*, 2005). Body fatness is the key factor regulating adipose tissue expression of leptin as well as circulating plasma leptin concentrations (Frederich *et al.*, 1995; Blache *et al.*, 2000; Chilliard *et al.*, 2005). Leptin concentrations were different between Genotypes in the experiment when the Genotypes differed in body fatness.

Leptin was associated with nutrition and Genotype, in the experimental cattle, and there were strong associations with body fatness. For all Genotypes there was a significant association between both pre- and post-calving leptin concentrations and pre-calving measures of P8 fat depth, further supporting the hypothesis that leptin concentrations are related to adiposity. The association of leptin concentrations with body fatness has been reported extensively by several authors (Frederich *et al.*, 1995; Howie, 1999; Delavaud *et al.*, 2000; Ciccioli *et al.*, 2003; Geary *et al.*, 2003; Leon *et al.*, 2004; Chilliard *et al.*, 2005; Kokkonen *et al.*, 2005; Lents *et al.*, 2005).

Body fatness, or the adiposity of an animal, reflects its nutritional history but circulating leptin concentrations have been shown to be regulated not only by degree of adiposity but also by energy intake level (Delavaud *et al.*, 2002). Block *et al.* (2001) showed that an energy deficit in periparturient dairy cows causes a sustained reduction in plasma leptin. They reported that the plasma concentration of leptin was positively correlated with blood parameters that are reflective of energy balance, namely plasma concentrations of insulin and glucose. Leptin was also negatively correlated with plasma concentrations of GH and NEFA, elevated concentrations of which are also indicative of a negative energy balance. Chilliard *et al.* (2005) reported that although leptin was strongly regulated by body fatness, leptinaemia is higher after underfeeding or during lactation, supporting the hypothesis that leptin concentrations are reflective of energy balance. Similar conclusions regarding leptin and energy balance have been reached by other researchers (Tokuda *et al.*, 2002; Liefers *et al.*, 2003b; Chelikani *et al.*, 2004; Meikle *et al.*, 2004; Konigsson *et al.*, 2008).

In the current experiment several results point to an association of leptin with energy balance. First, in 2008 animals on low-nutrition had significantly lower plasma leptin concentrations than those on high-nutrition both pre-and post-calving (see Figure 6-5 and Figure 6-13). It was shown in Chapter 5 that fewer MJ ME were available to the cows on the low-nutrition treatment during the breeding season in that year and mean leptin concentrations were reflective of the energy supply difference between the high- and low-nutrition treatments. Furthermore, the interaction between days-post-calving and nutritional treatment indicates that leptinaemia increased more quickly on the high-nutrition treatment post-calving than on the low-nutrition treatment (see Table 6-4). Although the change in serum leptin post-calving was affected by the change in P8 fatness whereby as the rate of fat accretion increased, so did leptin concentrations (see Table 6-4). This result suggests an indirect link to energy supply because fat accretion relies on the supply of adequate, if not excess energy in an animal in a particular physiological state.

The increased energy demand of lactation would have led to lower leptin concentrations in the lactating animals (Figure 6-14) but as the breeding season progressed and energy became more available through an improvement in pasture quality, leptinaemia increased significantly Table 6-3). Notably the only effect of

Genotype on leptin concentrations in either year was that Fat animals had more circulating leptin than Lean animals pre-calving in 2007, a result readily explained by the difference in body fatness.

Leptin was correlated with the blood metabolites that are indicators of energy balance. In the experiment there were significant correlations between leptin and BHB, and leptin and insulin (see Table 6-5). It has been shown that energy restriction decreased concentrations of insulin in heifers (Harrison and Randel, 1986; Yelich *et al.*, 1995) and BHB concentrations post-calving reflect the energy balance of the cow (Ingvartsen and Andersen, 2000). Block *et al.* (2001) showed that leptin concentrations are positively correlated with insulin and glucose and negatively correlated with GH, BHB and NEFA. The low serum leptin concentrations post-calving in dairy cows are directly associated with the negative energy balance, a consequence of the high energy demands of lactation (Block *et al.*, 2001).

The hypothesis that leptin is associated with energy supply as well as fatness is supported by this experiment. However, although leptin appears to be a reliable predictor of body condition it was not possible to identify a critical concentration of leptin at which productivity (in terms of fertility) was compromised. This was mainly owing to the lack of significant differences in our fertility parameters DTC (see 5.5.1) and PPAI (see Section 4.5). The lack of any difference in measured DM intake (see 5.5.5) meant that it was not possible to explore the previously reported associations between leptin and feed intake (Zarjevski *et al.*, 1993; Mercer *et al.*, 1997; Henry *et al.*, 1999; Ingvartsen and Boisclair, 2001; Morrison *et al.*, 2001).

Measurement of plasma NEFA showed that fatter animals tended to have higher circulating NEFA concentrations than leaner animals but this effect was only evident in 2007; NEFA was not measured in 2008. Financial constraints required that fewer metabolites could be analysed and as BHB was shown to be better associated with fatness and nutrition it was decided not to measure NEFA in the second year.

It appears that NEFA was associated with feed efficiency because NEFA concentrations were higher in high-NFI cattle. This result differs from two studies (Richardson *et al.*, 2004; Kelly *et al.*, 2010) in which NEFA concentrations were higher in low-NFI animals. Those authors (Richardson *et al.*, 2004; Kelly *et al.*, 2010) attributed that to the greater fat content in high-NFI animals and therefore a greater incorporation of triglycerides into their fat cells, as well as a greater energy requirement by muscle due to increased muscle turnover. It is hypothesised that high-NFI animals have higher tissue energy requirements, are more susceptible to stress and utilise different tissue substrates (partly as a consequence of differences in body composition) to generate energy required to respond to exposure to a stressful stimulus. This would lead to a greater uptake of mobilised fat and a lower plasma concentration in an animal at rest and possibly a greater capacity to mobilise fat during stress events.

Another postulated reason for the current result is that NEFA measurements were not a measure of NEFA concentrations in a resting animal but more an indicator of the extent to which NEFA was mobilised during a stressful event. This result would therefore be dependent on the amount of fat available to mobilise and hence the increased concentration of NEFA in fatter animals. Each group was walked for at least 20 minutes prior to the collection of blood samples. This degree of physical activity and unavoidable stress caused by mustering in yards and restraint for bleeding would have induced a short term, adrenalin-induced, hormone-sensitive lipase-regulated, elevation of NEFA (Veerkamp *et al.*, 2003). This short term stress response was therefore more a reflection of the capacity of fat animals to mobilise greater amounts of NEFA than leaner animals, than a reflection of maintenance requirements for fat mobilisation. For this reason NEFA was not measured in 2008 and instead BHB was used as the better indicator of energy status and requirement for alternative energy sources such as oxidation of NEFA to ketones.

The results of pre- and post-calving IGF-1 concentrations revealed a less clear cut reflection of energy balance than was expected and were more difficult to interpret. Many have reported that IGF-1 is related to energy balance; animals in positive energy balance have higher IGF-1 concentrations than those in negative energy balance (Spicer *et al.*, 1990; Yelich *et al.*, 1995; Yelich *et al.*, 1996; Konigsson *et al.*, 2008). IGF-1 has been shown to be also associated with BCS and therefore fatness (Richards *et al.*, 1991; Roberts *et al.*, 1997; Leon *et al.*, 2004; Lake *et al.*, 2006; Kelly *et al.*, 2010). In the current experiment IGF-1 concentrations were affected by both nutrition and Genotype (Fat vs. Lean), but only pre-calving in 2007. Lean animals had lower IGF-1 concentrations than Fat animals. Notably there were also significant differences in the fatness (amount of fat measured by US at the P8 site) of the Fat and Lean animals at this time (see Section 3.5.2). The depth of fat at the P8 site is considered a suitable indicator of BCS and therefore these results were consistent with Leon *et al.* (2004) who found that IGF-1 concentrations decreased as BCS declined ($P < 0.001$). These researchers also showed that IGF-1 concentrations increased significantly during periods of weight and fat gain. The influence of fatness on IGF-1 was evident in the pre-calving measure where P8 fat depth was significantly associated with pre-calving IGF-1 concentrations ($P < 0.001$), across all Genotypes, in both years of the experiment. In terms of energy supply, animals on a lower plane of nutrition pre-calving in 2007 had lower IGF-1 concentrations than animals on a high plane of nutrition. It was clearly shown in Figure 3-4 that a reduced energy supply caused a reduction in adiposity in the experimental animals and it is therefore difficult to determine whether the difference in IGF-1 pre-calving in 2007 was due to a difference in the supply of energy or the difference in fatness of the Genotypes, and it is probable that both of these influenced IGF-1 concentrations.

Post-calving the relationship between IGF-1 concentrations and fatness or nutrition was not clear cut. There were no main effects of nutrition or Genotype on IGF-1 concentrations in either year of the experiment. The results from this experiment showed that P8 change, as a reflection of BCS change post-calving, was not correlated with either mean post-calving IGF-1 (see Table 6-6) or the change in IGF-1 post-calving (see Table 6-5), nor was there any effect of pre-calving P8 fat depth on mean post-calving IGF-1 concentrations (see Table 6-3). This suggests little relationship between post-calving IGF-1 and BCS in the experimental cattle, which is contrary to the findings of Vizcarra *et al.* (1998) who described a significant relationship between pre-calving BCS and post-calving IGF-1, GH and insulin. The result also differs from the results of the pre-calving analysis which showed a significant relationship between fatness and IGF-1 concentrations.

The absence of any effect of nutrition on IGF-1 concentrations post-calving was unexpected because of the recognised association between energy supply and IGF-1. Similarly, no association between nutrition and mean post-calving insulin concentrations was found, although it was expected that insulin concentrations would reflect the negative energy balance associated with lactation and the low-nutrition treatment. However, IGF-1 and insulin were correlated, a finding that has been reported before in beef cattle (Bishop *et al.*, 1994; Vizcarra *et al.*, 1998). This suggests that although no nutritional effect was evident, the physiology of the IGF-1 and insulin response in the experimental cattle was not different to that that has been reported in the literature (Yambayamba *et al.*, 1996; Bossis *et al.*, 2000; Lake *et al.*, 2006).

There was no effect of Genotype on mean post-calving GH or insulin. The liver produces IGF-1 in response to GH stimulation (Cohick *et al.*, 1996) but feed restriction in animals often leads to a decline in circulating IGF-1 despite increases in GH, particularly post-calving. This is referred to as the "uncoupling" of the IGF-1-GH axis (Yambayamba *et al.*, 1996; Bossis *et al.*, 2000; Lake *et al.*, 2006). The results from the current experiment show that IGF-1 concentrations were not correlated to GH concentrations post-calving, suggesting that this uncoupling was taking place in the experimental animals. Furthermore, animals on low-nutrition did have higher mean post-calving GH concentrations than high-nutrition animals but IGF-1 was not affected by nutrition. Insulin was also negatively correlated to GH post-calving ($r = -0.2297$), a result which further goes to show that there is a partial uncoupling of the IGF-1-insulin-GH axis.

It is postulated that the absence of an IGF-1 response to high GH concentrations post-calving is possibly due to the loss of hepatic responsiveness to GH in energy-restricted animals. It has been suggested that the reason for this uncoupling of the GH-IGF-1 axis is that in an energy-restricted state there is a reduction in hepatic GH binding sites (Breier *et al.*, 1988). The high circulating concentrations of GH in energy-restricted animals are thought to be due to a decrease in the negative feedback of IGF-1 in the hypothalamus, resulting in increased GH synthesis (Kirby *et al.*, 1993). It has also been reported that cows with a high genetic merit for yield have high concentrations of GH (Hart *et al.*, 1978; Lukes *et al.*, 1989; Westwood *et al.*, 2000) which agrees with the results of the current experiment where Lean animals had higher GH concentrations than Fat animals. In Chapter 5 it was demonstrated that the Lean animals had significantly higher estimates of carcass yield, and blood parameters from these animals were reflective of decreased energy balance.

A possible reason for the absence of a nutrition effect on mean post-calving IGF-1 or insulin is that the energy balance changed during that time. Nutrition at both treatment levels improved as the season progressed owing to the increasing quantity and better quality of available pasture, and there was a corresponding increase in IGF-1 concentrations over that time. This is identified in the significant effect of days-post-calving on IGF-1, in that IGF-1 concentrations increased with the number of days-post-calving (see Table 6-2, $P < 0.001$). Post-calving IGF-1 figures were predicted averages over this period so perhaps the increase in IGF-1 that would correspond to improving quality and increasing quantity of feed is blunting any point differences at different times in the post-calving period, leading to an absence of any nutritional effect in the analysis. It is also possible that the difference in nutritional treatments was not as great as the change in IGF-1 over the calving period. There could have been a large degree of variation not only between animals but also between plots which would account for the lack of significance. It would take further research and possibly an increase in the number of times animals were sampled after calving to fully elucidate the reasons for the absence of a nutritional effect, something that was beyond the financial and practical bounds of the experiment.

The results of the assay of pre- and post-calving acetate concentrations were difficult to interpret. Plasma acetate is postulated to be reflective of the endogenous production of acetate in response to increased fatty acid oxidation in animals on a reduced plane of nutrition (Annison and White, 1962). Their results were consistent with the hypothesis that the oxidation of free fatty acids contributes substantially to the entry into plasma of endogenous acetate in sheep. Acetate measured in animals on low-nutrition is reflective of endogenous production as well as rumen-produced acetate, whereas in well-fed animals the total plasma pool of acetate is derived mainly from rumen production (Annison and White, 1962).

Some of the results of the current experiment support this, with energy supply being the factor that appears most to influence acetate concentrations. In both years low-nutrition animals had higher acetate concentrations than the high-nutrition treatment animals, and in 2008 acetate concentrations increased faster on the low-nutrition treatment animals than on the high-nutrition treatment animals post-calving. This would indicate that as animals progressed through their lactation and the energy deficit became large through a reduced energy supply and the increased burden of lactation, acetate concentrations rose accordingly. Supporting this was the finding that post-calving acetate concentrations were higher in lactating compared to non-lactating animals (see Section 6.5.2). This result mirrors the association between lactation, energy balance and other blood parameters such as post-calving BHB, leptin and NEFA (see Figure 6-9 and Figure 6-12). These parameters were all affected by lactation and energy balance and support a link between fat mobilisation, energy balance and acetate concentrations.

There was a negative correlation of acetate concentrations to the change in P8 fatness post-calving (see Table 6-6), which could indicate an indirect link to energy balance. Fat reserves are depleted when they are mobilised to meet an energy demand, and with reducing fatness there were increased plasma acetate concentrations, supporting the association between energy balance and acetate concentrations. Additionally, acetate was associated with body condition pre-calving whereby fatter animals had lower acetate concentrations than leaner animals (see Section 6.5.1).

However, apart from the pre-calving 2007 result, there was no significant effect of nutrition on acetate concentrations. An explanation for this could be that the ratio of endogenous vs. rumen acetate is different on different nutritional treatments and may remove any significant difference between the two treatments (van Houtert, 1993). Associations with other blood parameters seem a more reliable method of supporting the hypothesis. The conclusion reached from the measurement of acetate in this experiment is that it is to some extent associated with energy balance, but is not a particularly sensitive measure of energy balance, feed intake or body condition. It was certainly not a predictor of Genotypic difference and cannot be used as a marker for feed efficiency.

As hypothesised, the significant associations between experimental Genotype and blood parameters were generally explained by the fatness of the animals. Fat animals were fatter than Lean animals and high-NFI animals were fatter than low-NFI animals. These differences resulted in differences in blood parameter concentrations, particularly BHB, leptin, NEFA and IGF-1. The Genotype effects on blood parameters were notable only in parameters that reflect fatness or energy balance such as insulin, glucose, leptin, GH and IGF-1.

Another major hypothesis was that no single blood parameter could be used as a marker for feed efficiency. Feed-efficient animals and Lean animals had similar blood physiology patterns, as did Fat and high-NFI animals, but measuring of the parameters could not be used a tool to distinguish them. Blood parameters, although reflective of the fatness and energy balance in a cow, were not reflective of the experimental traits themselves and could not be used as physiological markers for them.

Others have investigated concentrations of blood parameters as possible markers for feed efficiency in beef cattle (Richardson and Herd, 2004; Wood *et al.*, 2004; Nkrumah *et al.*, 2007b; Kelly *et al.*, 2010). It was initially postulated that a correlation existed between circulating IGF-1 concentrations and NFI (Stick *et al.*, 1998; Wood *et al.*, 2004; Moore *et al.*, 2005), until the relationship was explored further and it was found that IGF-1 correlated only with NFI measured in very young and growing animals (Johnston, 2007). Kelly *et al.* (2010) reported that in samples collected from beef heifers at the end of an individual feed test, there was a positive correlation (r = 0.24) between NFI and IGF-1 concentrations in that more efficient animals had lower circulating IGF-1 concentrations, but on the whole there was no relationship with NFI in their study. Their result is similar to the results reported here. Genotype (high- vs. low-

NFI) did not affect IGF-1 concentrations in either year of the experiment either pre- or post-calving, suggesting no relationship between feed efficiency and IGF-1. The experimental animals were all past the weaning stage when both IGF-1 and NFI were measured so this result was expected. The findings concur with Johnston (2007) who reported that IGF-1 and NFI have a genetic correlation of r = -0.22 (± 0.16) and Lancaster *et al.* (2008) who found that IGF-1 selection had no effect on NFI in beef heifers. Their conclusion was that it was effectively impossible to use IGF-1 as any kind of predictor of NFI and the results of the current experiment concur with this.

There were no strong marker/ trait associations identified in this experiment that support the use of a single blood parameter for a marker for feed efficiency. For instance where others have identified BHB as possibly being associated with NFI, the current results did not. Richardson and Herd (2004) reported a positive correlation at weaning between BHB and NFI (r = 0.55) and Kelly *et al.* (2010) reported a positive correlation of 0.37 but few other reports have investigated the association between NFI and BHB. Similarly some studies have reported a greater systemic insulin concentration in high-NFI cattle (Richardson *et al.*, 2004) but there was no such relationship in the current study or in Kelly *et al.* (2010).

The experimental results also show no relationship between serum leptin and feed efficiency, given the lack of any Genotype (high- or low-NFI) effect on leptin concentrations. Kelly *et al.* (2010) also reported there was no correlation between leptin and NFI. This differs from results reported by Richardson *et al.* (2004) who described a correlation (r = 0.31) between NFI and leptin concentrations and postulated that it was in line with the increase in fatness of the less efficient steers in their studies. Given the recognised difference in fatness between high and low feed-efficient animals (Herd and Bishop, 2000; Richardson *et al.*, 2001; Basarab *et al.*, 2003; Kelly *et al.*, 2010), it is a little surprising that there are not stronger associations between leptin and feed efficiency. It would be valuable to measure feed efficiency in animals with divergent serum leptin concentrations.

Although it was hypothesised that different blood parameter concentrations would be associated with different measures of the PPAI, the results from the experiment did not fully support this. Studies have investigated the relationship between post-calving blood metabolites and resumption of postpartum oestrus (Rutter *et al.*, 1989; Spicer *et al.*, 1990; Roberts *et al.*, 1997; Spicer *et al.*, 2002) and the findings differ somewhat. Spicer *et al.* (2002) described that postpartum serum IGF-1 concentrations were associated with BCS but not the number of days to the first medium or large follicle postpartum, and Roberts *et al.* (1997) concluded that serum IGF-1 concentrations 2 weeks postpartum were indicators of the capacity of energy-restricted cattle to resume cycling after parturition. It was difficult for a couple of reasons to test the association between IGF-1, or any other blood parameter, and the resumption of oestrus in the experimental cattle for a couple of reasons. First, there was no accurate measure obtained of the timing of the resumption of oestrus (see Chapter 4). Secondly, there was no difference in DTC detected between Genotypes in either year of the experiment (see Figure 5-3). Nutrition, however, affected not only days-to-calving but post-calving IGF-1 concentrations as well. Thus, the result supported the accepted principle that BCS as well as serum IGF-1 concentrations have a role to play in the timing of the postpartum resumption of oestrus (Rutter *et al.*, 1989; Spicer *et al.*, 1990; Roberts *et al.*, 1997; Butler, 2000; Spicer *et al.*, 2002).

It has been suggested that BHB concentration is the best predictor of the onset of the oestrous cycle post-partum in cattle (Reist *et al.*, 2000) and the experimental results partially supported this. Reist *et al.* (2000) showed that animals with high postpartum concentrations of BHB had a delayed postpartum resumption of oestrus and that it was the maximal concentrations of BHB that were the best predictor of the onset of the oestrous cycle. Oikonomou *et al.* (2008) reported that parameters that are usually negatively correlated with energy balance, such as BHB and NEFA, measured at peak lactation, had the highest genetic correlation with future reproductive performance. Once again it was difficult to draw conclusions about the impact of post-calving BHB on the reproductive cycle because of the lack of difference in DTC between Genotypes. However, the results from the current experiment do show that BHB concentrations were higher on average in the low-nutrition treatment and in leaner animals. This concurs with the accepted principal that BHB is a blood indicator of negative energy balance and it has been widely reported that a negative energy balance is associated with prolonged postpartum anoestrus (Richards *et al.*, 1989; Randel, 1990; Lucy *et al.*, 1991; Murphy *et al.*, 1991; Bergfeld *et al.*, 1994; Gutierrez *et al.*, 1997; Wettemann and Bossis, 1999; Yavas and Walton, 2000; Rhodes *et al.*, 2003). It follows that elevated BHB postpartum it is likely to be associated with a delayed return to oestrus. It was concluded that in the present experiment there was not enough power to detect differences in DTC between Genotypes and to attribute statistical significance to the trend that Lean and low-NFI animals have longer DTC that Fat or high-NFI animals. However, there was a significant increase in DTC in the low-nutrition treatment in 2008 (result discussed in section 5.6.2). Therefore, as BHB was higher on the low-nutrition treatment it is concluded that BHB can be associated with post partum resumption of oestrus due to the effect of low-nutrition on DTC.

This experiment used a one-off pre-calving sample and up to eight post calving samples over a breeding season that lasted five months. Some animals were sampled only once post-calving because the lateness of their calving date. Although the results tend to be consistent with other studies, some effects were not evident in these results. A possible reason for this is that although samples were taken at regular intervals during the breeding season, the sampling was done no more frequently than once a fortnight.

Every effort was made to sample the animals within a two hour time frame at the same time of day but no account could be taken of different levels of gut fill, stress, timing of feeding or suckling events, or the fact that blood parameter concentrations are not static throughout the day. Far more intensive sampling would be required to be more specific about the detailed fluctuations of blood parameters within a day, something that was beyond the practical and financial scope of this experiment. The results give a broad characterisation of the physiological picture of animals of differing Genotypes on different levels of nutrition but conclusions about the intricate interactions between hormones and metabolites is not possible.

6.7 Conclusions

It was hypothesised in Chapter 5 that Lean animals and more feed-efficient (low-NFI) animals may be less productive in times of reduced energy supply. This was shown not to be the case; there were no differences in productivity detected between the Genotypes (see 5.6.3) on the low-nutrition treatment. However, nutritional treatment did impact on efficiency across all Genotypes (see Section 5.5.5.). Measured blood parameters appeared to be reflective of the fatness of the animals as well as their energy balance. Differences in concentrations of circulating blood parameters differed for the different Genotypes when there was a difference in fatness between the Genotypes. No single measurement of a blood parameter could be used to distinguish one Genotype from another and therefore use of blood parameters for MAS is probably not possible because of the complex relationships among measured parameters, fatness, energy balance and nutritional treatment. Differences in fertility between Genotypes were also not reflected in differences in blood parameter concentrations. Although some measured parameters were associated with an increase in DTC and therefore associated with decreased productivity, the associations are in line with the nutritional treatments in the experiment, not the specific Genotypes. This experiment has found no unexpected differences in the physiology of the Genotypes. Beef producers can be confident that selection for increased feed efficiency and leanness is not associated with selection for a change in, or a compromise to, normal animal physiology.

CHAPTER 7. CHAPTER SINGLE NUCLEOTIDE POLYMORPHISMS IN THE BOVINE LEPTIN GENE AND THEIR ASSOCIATION WITH CARCASS AND EFFICIENCY TRAITS, AND PRE- AND POST-CALVING ENDOCRINE PROFILES, IN COWS SELECTED FOR A DIVERGENCE IN FATNESS OR FEED EFFICIENCY

7.1 Introduction

MAS for economically important traits in cattle has the potential significantly to alter the rate of genetic improvement, particularly when the marker-trait association is strong. The principle behind MAS is that polymorphisms such as mutations, insertions or deletions in the cattle genome are often marked by a Single Nucleotide Polymorphism (SNP). If SNPs are statistically significantly associated with a desirable phenotype in a population of animals they are then termed Quantitive Trait Loci (QTL). It was suggested by Edwards and Page (1994) that total genetic gain, particularly in the first three years of selection, could be very high, depending on the model and strength of marker-trait association. They stated that linkage distance between markers and QTLs was the factor which most limited the responses from MAS. Marker/trait associations are the focus of much research in Australia at the present time because of the potential dramatically to improve the ability to select for desirable traits in beef cattle. The aim is to include in the national genetic evaluation scheme Breedplan molecular EBVs which consist in part of results obtained from gene marker evaluations of beef cattle.

Several studies over the past few years have explored the association between SNPs in the exon and promoter region of the bovine leptin gene and various carcass, growth and production traits (Fitzsimmons *et al.*, 1998; Buchanan *et al.*, 2002; Liefers *et al.*, 2002; Liefers *et al.*, 2003a; Crews *et al.*, 2004; Nkrumah *et al.*, 2004b; Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005; Schenkel *et al.*, 2005; Lusk, 2007). Relationships between leptin SNPs and fatness, lean meat yield, eye muscle area, marbling, growth, ultrasound back fatness, feed intake, NFI and serum leptin concentrations have been established but their associations with these traits have not been consistently verified across studies (Schenkel *et al.*, 2005). Most of the studies have been undertaken on North American cattle populations (Buchanan *et al.*, 2002; Nkrumah *et al.*, 2004b; Nkrumah *et al.*, 2005; Schenkel *et al.*, 2005; Lusk, 2007) and they all reached similar conclusions about the associations between SNP and carcass, growth and production traits which are described in detail in Section 1.4.2. However, when Barendse *et al.* (2005) investigated a SNP in a large population of Australian cattle it was concluded that marker-trait associations existing in North American cattle populations may not exist in Australian cattle populations. Only one of the North American studies included female cattle in the analysis (Schenkel *et al.*, 2005) and thus little information exists about marker-trait associations in breeding cattle. Identification of strong SNP/trait associations in Australian cattle and their relationships to carcass and efficiency traits has the potential considerably to enhance the ability of producers to select for desirable and economically beneficial, heritable traits in their cattle.

7.2 Aims

The aims of this experiment were to:

1. genotype the cattle used in the current experiment with respect to the SNPs UASMS1 and UASMS2 in the promoter region and E2FB and E2JW in the exon region of the bovine leptin gene;
2. identify associations between the SNPs and the traits in which the experimental animals were selected for a divergence, namely a divergence in fatness or a divergence in feed efficiency;
3. identify associations between the SNPs and pre- and post-calving blood parameters in the experimental cattle;
4. use genotyping in an expanded population of cattle with EBV data for NFI to validate the results for SNP/trait association for the E2JW SNP.

7.3 Hypotheses

It was hypothesised that:

- the frequency distribution of the four leptin SNPs genotyped in VRC experimental cattle would be similar to that described in studies of North American populations of cattle;
- there would be associations between the SNP identified in our experimental cattle and carcass and efficiency traits, but that these associations would differ from those reported in studies of North American populations of cattle;

- there would be associations between SNP and pre- and post-calving blood parameters, but these associations would differ from those reported in the studies of North American populations of cattle.

7.4 Materials and methods

7.4.1 Animals

The animals used in this experiment are as described in Chapter 2 (VRC experimental animals). Table 2-1 in that chapter outlines the experimental design and allocation of animals to the experiment. All the animals in the 1st cohort and only the Trangie animals in the 2nd cohort are included in this experiment. Funding constraints meant that we were unable to identify SNP in the Industry animals in the 2nd cohort.

Data were acquired from an additional 169 cattle, both male and female, originating from the Trangie NFI-selected herd to increase the number of cattle genotyped for the E2JW SNP. These animals varied in age and were tested for feed efficiency at several locations. SNP data were provided for these animals and in particular data for the E2JW SNP were used in this analysis. NFI EBV data, done in December 2008, were sourced for the VRC experimental animals as well as the aforementioned Trangie animals and used in analysis to examine the effect of the E2JW SNP on NFI EBV.

7.4.2 Collection of blood samples

Blood samples from all animals were collected via the methods described in Section 2.3.3 and frozen in 9 ml EDTA tubes (SARSTEDT Australia Pty. Ltd. 16 ParkWay, Technology Park South Australia, 5095). These samples were submitted to laboratories for DNA extraction and SNP analysis. Samples were collected at the end of the individual feed test which established actual NFI for each individual animal. The

Industry animals, which did not undergo an individual feed test, were sampled at the same time. The age of the animals at this stage ranged from 12 – 18 months.

7.4.3 DNA extraction and SNP analysis

DNA extraction was done by Saturn Biotechnology, Murdoch University, South Street, Murdoch, WA, 6150. DNA was extracted using the Edna HiSpEx™ Blood Kit (Saturn Biotechnology, Murdoch University, South Street, Western Australia). Protocols are attached in Appendix 2.

The SNP analysis for the first cohort of VRC experimental animals was done by Saturn Biotech using pyrosequencing techniques. A PSQ^{TM}96MA System (Pyrosequencing AB., Roche Molecular Systems and F. Hoffmann-La Roche Ltd.) was used to genotype the experimental animals in the first cohort.

SNP analysis for the 2nd cohort was done by Biosciences Research Division, Department of Primary Industries, 1 Park Drive, Bundoora, Victoria 3083, Australia. This laboratory used the "GoldenGate" system from Illumina Inc, USA (www.illumina.com) and all product literature can be viewed at www.illumina.com/pagesnrn.ilmn?ID=70#45.

7.4.4 Statistical Analysis

The following statistical models were fitted to test the hypotheses in Section 7.3 using the SNP results from this experiment and SNP results from Trangie sourced animals:

1. *For each leptin SNP, comparison of allele distribution between experimental genotypes*.

   In this analysis counts in each genotype and for each SNP allele were fitted to a log linear model with a Poisson error distribution and the linear predictor:

   constant + SNP + Genotype + SNP.Genotype

   The genotype effect was subdivided as described in Section 2.10.1.

2. *Comparison of the frequency of the SNP alleles in VRC experimental animals to frequency of SNP allele reported in North American studies (Kononoff et al., 2005; Nkrumah et al., 2005).*

   This was done using a Pearson Chi-Squared test.

3. *Evaluation of associations between leptin SNP and the dependent variables (carcass and efficiency traits) before experimental treatment effects were imposed*.

   For this analysis the models were:

   constant + dam birth date + SNP

4. *Evaluation of associations between SNP and the dependent variables (carcass and efficiency traits) after experimental treatment effects were imposed.*

   The models used for these analyses were more complex and included treatment effects and various covariates. The model was defined as:

   constant + location + cohort + height + calving date + line + nutrition + SNP + line.nutrition + line.SNP + nutrition.SNP + line.nutrition.SNP.

5. *Evaluation of associations between E2JW SNP and efficiency traits (NFI EBV) in Angus cattle sourced from the Trangie research herd*

   As the numbers used in the analysis of the E2JW SNP in the VRC experimental cattle were reasonably small (37) it was useful to look at the distribution of the SNP and its alleles, as well as its association with efficiency traits in a larger population. For this we used animals originating from the Trangie research herd and added them to the data set. In this analysis we used NFI EBV instead of actual NFI in order to compare animals that were tested for NFI at different locations.

   The model was defined as:

   constant + SNP

   As the dependent variable is an EBV no adjustment is made for date of birth as this is taken into account in the generation of the EBV.

The models described above were fitted to data from VRC experimental animals and data from the Trangie sourced animals using the regression procedures in GenStat 11th edition (VSN International Ltd, Hertfordshire, UK). Hierarchical tests (Type I sums of squares) were used and a 5 % level of significance was used to assess the significance of terms in the models.

7.5 Results

Table 7-1 is a summary of the leptin SNP analysis in the VRC experimental animals, categorised by experimental Genotype, i.e. Fat, Lean, high-NFI or low-NFI, and SNP genotype frequency. For each of the leptin SNP in this table the mutation involves a single nucleotide transition and thus the leptin SNP genotypes are either homozygous or heterozygous. As identified in previous papers (Schenkel *et al.*, 2005), for UASMS1, UASMS2 and E2FB the genotypes are CC, CT or TT but for the E2JW SNP the genotypes are AA, AT or TT. Analysis of the E2JW SNP was restricted to NFI animals because no data were available for industry animals.

Table 7-1: Percentage of leptin SNP Genotype (CC, CT or TT) for SNP (UASMS1, UASMS2, E2FB) and (AA, AT or TT) for E2JW SNP, total number of animals typed and allele %. Animals are categorised by experimental genotypes: Industry (Fat or Lean) and NFI (high-NFI or low-NFI).

UASMS1	**CC** %	**CT** %	**TT** %	**Total** count	**T** %
Fat	7%	50%	43%	28	68%
Lean	16%	59%	25%	32	55%
HiNFI	18%	51%	31%	39	56%
LoNFI	27%	52%	20%	44	47%

UASMS2	**CC** %	**CT** %	**TT** %	Total	**T** %
Fat	39%	54%	7%	28	34%
Lean	43%	57%	0%	28	29%
HiNFI	40%	48%	12%	42	36%
LoNFI	58%	39%	3%	38	22%

E2FB	**CC** %	**CT** %	**TT** %	Total	**T** %
Fat	36%	46%	18%	28	41%
Lean	18%	61%	21%	28	52%
HiNFI	5%	40%	55%	42	75%
LoNFI	17%	57%	26%	42	55%

E2JW	**AA** %	**AT** %	**TT** %	Total	**T** %
Fat	0	0	0	0	
Lean	0	0	0	0	
HiNFI	35	59	6	17	35%
LoNFI	75	25	0	20	13%

There was no difference in the distribution of the SNP genotype between the VRC experimental genotypes for SNPs UASM1, UASM2 or E2JW (Table 7-2). For the

E2FB SNP there was a significant difference in SNP genotypes between Industry and NFI animals (P =0.007) as well between high-NFI and low-NFI animals (P =0.014). There are significantly more homozygous TT genotype animals in the group of high-NFI than among the low-NFI animals (Figure 7-1) and there are significantly more animals with the T allele among the high-NFI animals (Table 7-1).

There was no difference between Fat and Lean Industry animals (P =0.313).

Table 7-2: Significance table (P-values) for distribution of SNP by comparing VRC experimental line and genotype.

	UASMS1	**UASMS2**	**E2FB**	**E2JW**
	P-value	P-value	P-value	P-value
Industry vs. NFI	0.184	0.324	**0.007**	**N/A**
Fat vs. Lean	0.269	0.241	0.313	N/A
HiNFI vs. LoNFI	0.434	0.131	**0.014**	**N/A**

The allele frequency distribution in VRC experimental animals was compared to the allele frequency distribution reported in North American studies (Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005) was analysed. The results of a Pearson Chi-Squared test are shown in Table 7-3. There was a significant difference in the allele frequency distribution of the E2FB and E2JW SNPs.

Table 7-3: Pearson Chi-squared test results for SNP in VRC experimental cattle compared to results in Kononoff *et al.* (2005) and Nkrumah *et al.* (2005)

SNP	**χ^2 – value**	**df**	**P – Value**
UASMS1	7.52	6	0.275
UASMS2	8.01	6	0.237
E2FB	21.9	6	**0.002**
E2JW	5.90	1	**0.015**

The significant difference between the high- and low-NFI for the E2FB SNP allele frequency (see Table 7-1), genotype count prediction and standard error for the E2FB SNP, is illustrated in Figure 7-1. There are significantly more homozygous TT genotype animals among the high-NFI animals than among the low-NFI animals. There are significantly more animals with the T allele within the group of high-NFI animals.

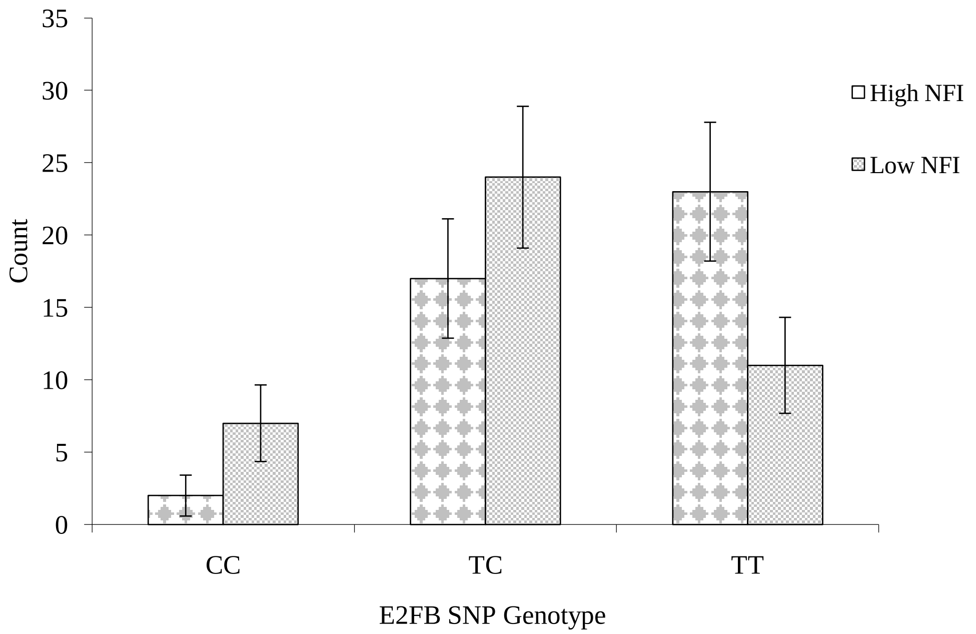


Figure 7-1: SNP genotype count prediction and standard error associated with the E2FB SNP in VRC experimental animals. Error bars represent standard errors.

The association was determined between SNP and carcass and efficiency traits post NFI test. Traits include actual NFI and US measures of P8 fat, rib fat, IMF and EMA, as well as the height of the animals. As the animals were different ages, date of birth was used as a covariate in the analysis.

Table 7-4 shows the level of significance for the covariate and the SNP, additive effect and dominance effect for each of the analysed traits. Date of birth was a significant covariate for all traits in all SNPs except for NFI. There was a significant association between NFI and P8 and the E2JW SNP ($P = 0.005$ and $P = 0.050$, respectively). The means for these traits in E2JW SNP allelic combinations are illustrated in Figure 7-2 and Figure 7-3. Figure 7-2 shows that A allele homozygote animals in the E2JW SNP have significantly lower actual NFI than the heterozygote or the homozygous T allele animals. A similar pattern exists whereby homozygous AA animals have a significantly lower ultrasound P8 measure than heterozygotes or homozygous TT animals.

Table 7-4: Significance level (P-values), additive effect (add) and dominance effect (dom) trait associations between date of birth (dob) covariate and SNP, carcass and efficiency traits in VRC experimental animals.

	UASMS1				**UASMS2**				**E2FB**				**E2JW**			
	dob	SNP	add	dom	dob	SNP	add	dom	dob	SNP	add	dom	dob	SNP	add	dom
NFI	0.581	0.422	0.024	0.729	0.947	0.100	**0.032**	0.942	0.684	0.065	**0.020**	0.986	0.250	**0.005**	**0.005**	N/A
post NFI test P8	**<0.001**	0.953	0.759	0.965	**0.001**	0.132	0.158	0.150	**<0.001**	0.076	**0.024**	0.852	0.014	**0.050**	**0.050**	N/A
post NFI test IMF	**<0.001**	0.941	0.934	0.736	**<0.001**	0.560	0.453	0.442	**<0.001**	0.371	0.160	0.973	**<0.001**	0.719	0.719	N/A
post NFI test EMA	**<0.001**	0.565	0.939	0.288	**<0.001**	0.409	0.748	0.196	**<0.001**	0.432	0.495	0.271	**<0.001**	0.991	0.991	N/A
post NFI test height	**<0.024**	0.236	0.100	0.689	**<0.001**	0.522	0.632	0.327	**<0.001**	0.088	**0.047**	0.333	**0.002**	0.392	0.392	N/A

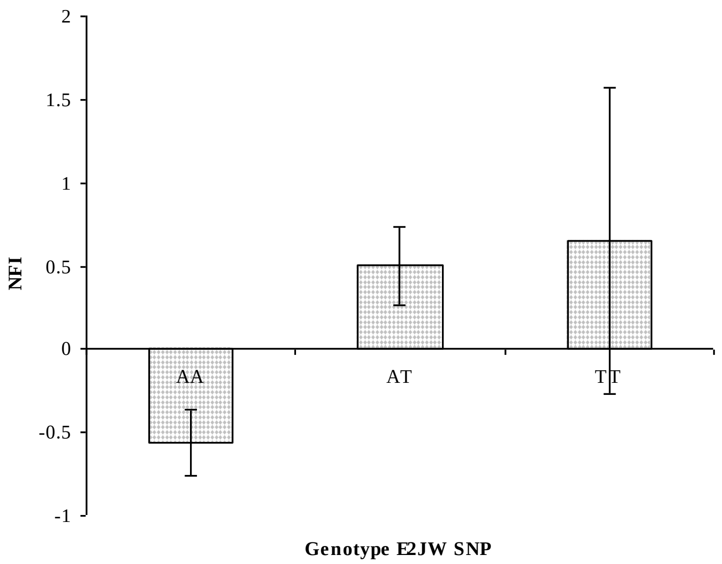


Figure 7-2: Mean NFI for AA, AT and TT genotypes of the E2JW SNP in the VRC experimental animals. Error bars represent standard errors.

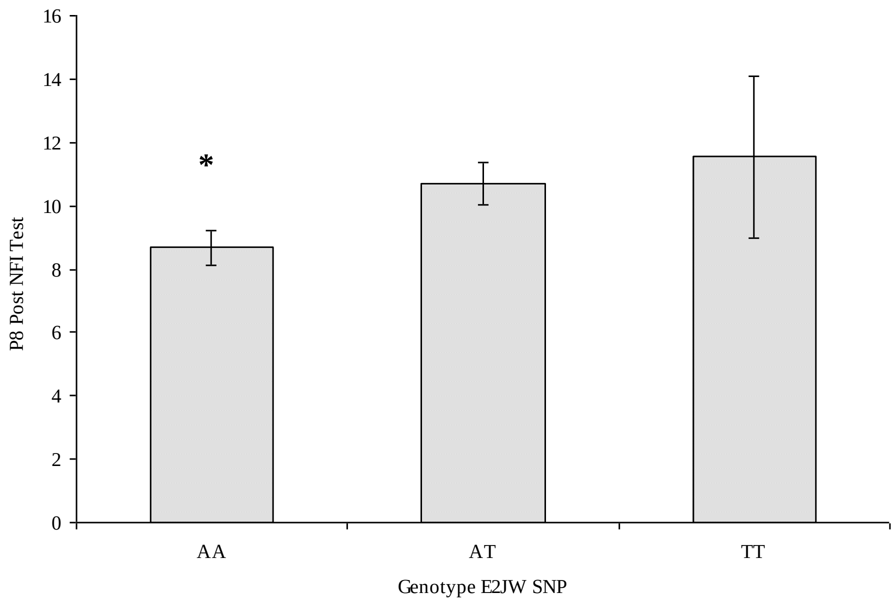


Figure 7-3: Mean ultrasound measured P8 fat (mm) depth for AA, AT and TT genotypes of the E2JW SNP in the VRC experimental animals. Error bars represent standard errors.

Table 7-5 shows the count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the data set that includes the extra Trangie Research Station sourced animals. The homozygous AA animals again had a significantly lower mean NFI EBV than the heterozygotes or the homozygous TT animals and this result is depicted in Figure 7-4. The distribution of genotypes within the SNP is similar to that detected in the VRC experimental population with the only 1% of animals being homozygous TT.

Table 7-5: Count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the data set including extra Trangie Research Station sourced animals.

	E2JW SNP			
Genotype	AA	AT	TT	F pr.
count	221	81	3	
mean NFI EBV	-0.036	0.315	0.417	**<0.001**

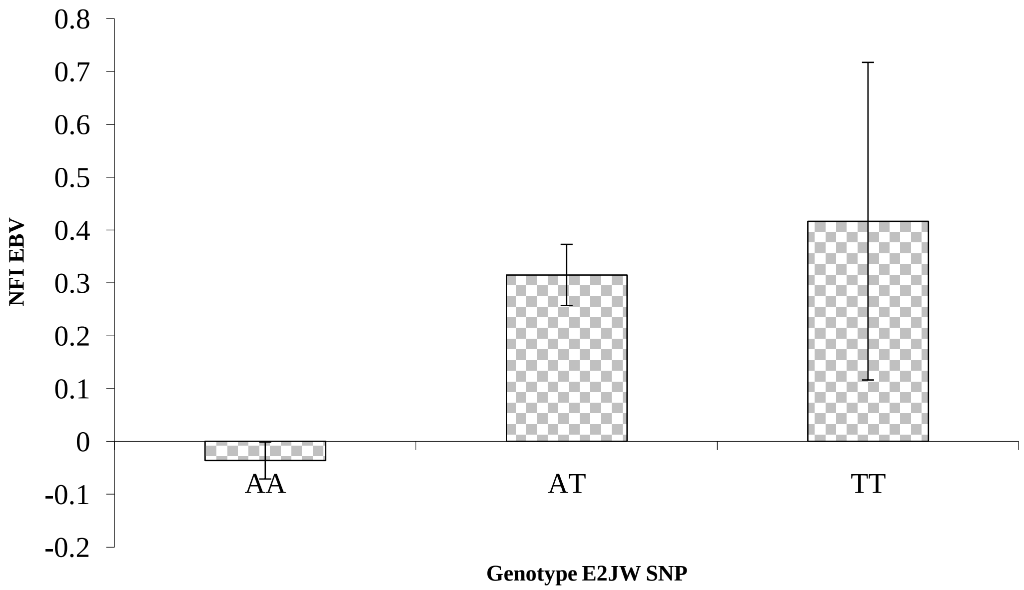


Figure 7-4: Mean NFI EBV categorised by E2JWE SNP Genotype (AA, AT or TT) in the expanded data set. Error bars represent standard errors.

An arbitrary classification into high-NFI (EBV > 0.5), average-NFI (EBV – 0.5 – 0.5) and low-NFI (EBV < - 0.5) was put in place. This was done to categorise animals into the top and bottom 10% (high or low efficiency animals) of the population tested, and the rest (average feed efficiency animals). This classification mimics the classification of the NFI animals into high- or low-NFI experimental Genotypes as was done for the VRC experimental animals. Table 7-6 shows the number of animals of each genotype within each category and the percentage of the total number of each allele within each category and Figure 7-5 is included to give an impression of the proportion of animals that would be misclassified if SNP were used to predict EBV category. For example, despite the significant association between AA animals and a negative NFI EBV, only 42% of animals with the AA genotype would be correctly classified as low-NFI.

Table 7-6: Number of animals (AA, AT or TT) and percentage of total within category (low-NFI, average-NFI or high-NFI).

	E2JW SNP Genotype					
	AA		**AT**		**TT**	
Category	count	%	count	%	count	%
low-NFI (< -0.5)	91	42	12	15	0	0
average-NFI (-0.5 - 0.5)	50	22	16	20	1	33
high-NFI (> 0.5)	80	36	53	65	2	67

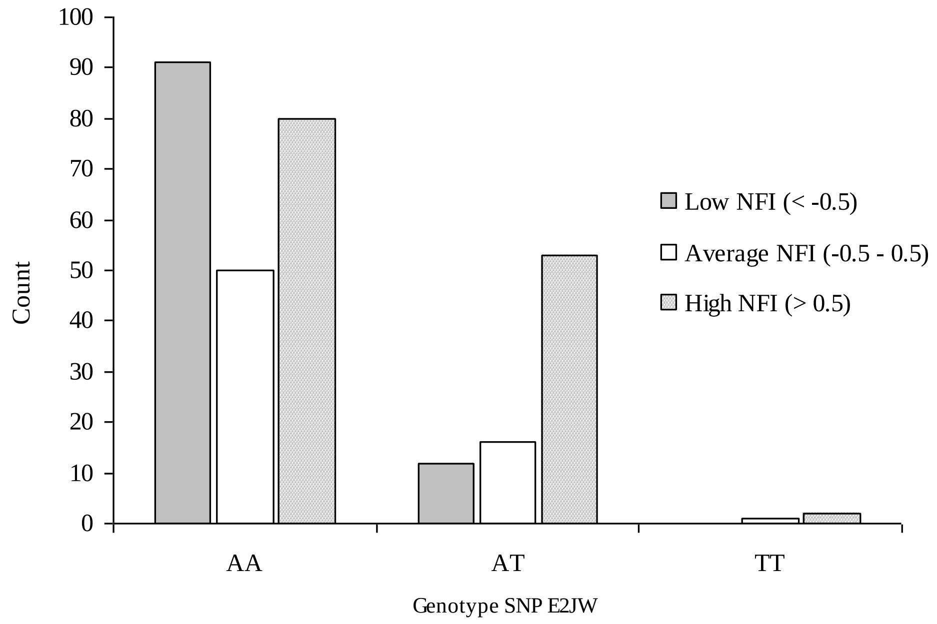


Figure 7-5: Number of animals of each genotype (AA, AT or TT) within category (high-, average- or low-NFI EBV).

The association between SNPs and pre- and post-calving US measures of P8 fat depth, EMA and IMF was determined. Table 7-7 shows levels of significance for each of the SNPs as well as additive and dominance effect/trait associations. There were no significant associations between SNP and any pre- or post-calving US measures. There was a significant additive effect of UASMS1 SNP on pre-calving IMF ($P = 0.024$). The effect was 0.6337 ± 0.28863 (results not displayed).

Table 7-7: Significance of associations (P-values) between UASMS1, UASMS2, E2FB and E2JW SNP, additive (Add) and dominance (Dom) effects for each SNP and pre- and post-calving ultrasound measures of EMA, IMF and P8 fat depth.

SNP	**EMA**		**IMF**		**P8**
	pre-calving	**post-calving**	**pre-calving**	**post-calving**	**post-calving**
UASMS1	0.452	0.867	0.073	0.652	0.929
add	0.212	0.907	**0.024**	0.518	0.742
dom	0.884	0.603	0.740	0.509	0.843
UASMS2	0.509	0.395	0.808	0.875	0.288
add	0.248	0.901	0.652	0.635	0.567
dom	0.883	0.173	0.337	0.837	0.138
E2FB	0.771	0.508	0.593	0.662	0.476
add	0.619	0.642	0.308	0.876	0.227
dom	0.602	0.287	0.999	0.372	0.857
E2JW	0.237	0.687	0.639	0.870	0.151
add	0.237	0.687	0.639	0.870	0.151
dom	N/A	N/A	N/A	N/A	N/A

The association between SNP and pre- and post-calving leptin, IGF-1, Insulin and GH concentrations was also determined. Table 7-8 shows levels of significance for each of the SNP/trait associations. UASMS1 and E2FB SNP were significantly associated with pre- and post-calving leptin concentrations but there was no significant association among any of the SNPs and ultrasound measures pre- and post-calving

Table 7-8: Associations between UASMS1, UASMS2, E2FB and E2JW SNP and pre- and post-calving concentrations of leptin, IGF-1, insulin and GH.

SNP	**leptin**		**IGF-1**		**GH**		**insulin**	
	pre-calving	post-calving	pre-calving	post-calving	pre-calving	post-calving	pre-calving	post-calving
UASMS1	**<0.001**	**<0.001**	0.772	0.259	0.274	0.882	0.956	0.941
add	**<0.001**	**<0.001**	0.799	0.212	0.183	0.738	0.873	0.732
dom	0.602	0.754	0.502	0.283	0.366	0.711	0.800	0.949
UASMS2	0.873	0.526	0.359	0.654	0.806	0.964	0.794	0.056
add	0.611	0.455	0.246	0.571	0.766	0.786	0.497	0.160
dom	0.909	0.392	0.770	0.466	0.557	0.985	0.983	0.055
E2FB	**<0.001**	**<0.001**	0.949	0.512	0.368	0.388	0.974	0.899
add	**<0.001**	**<0.001**	0.810	0.263	0.202	0.187	0.818	0.999
dom	**<0.001**	**0.002**	0.828	0.777	0.503	0.712	0.996	0.655
E2JW	0.758	0.840	0.655	0.798	0.231	0.211	0.585	0.652
add	0.758	0.840	0.655	0.798	0.231	0.211	0.585	0.652
dom	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Animals carrying the T allele for UASMS1 SNP had significantly higher pre- and post-calving leptin concentrations that the homozygous CC animals, with the homozygous TT having higher concentrations that the TC animals. Pre-calving concentrations are uniformly higher than post-calving. These results are illustrated in Figure 7-6.

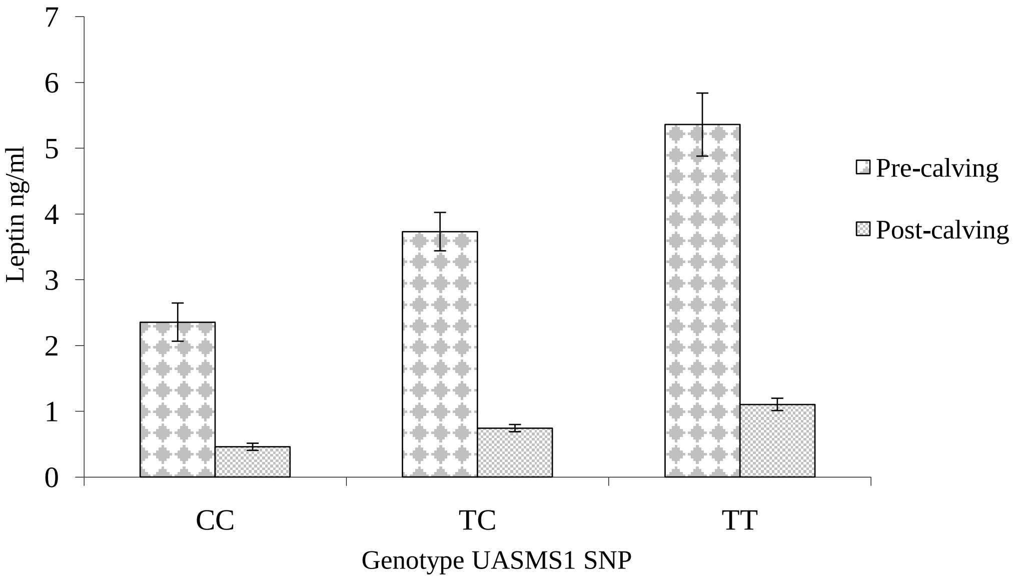


Figure 7-6: Mean pre- and post-calving leptin concentrations in VRC experimental animals with CC, TC or TT alleles of the UASMS1 SNP. All means differ significantly. Error bars represent standard errors.

Animals carrying the C allele for E2FB SNP had significantly higher pre- and post-calving leptin concentrations that the homozygous TT animals, with the homozygous CC having higher concentrations that the TC animals. Pre-calving concentrations are uniformly higher than post-calving. These results are illustrated in Figure 7-7.

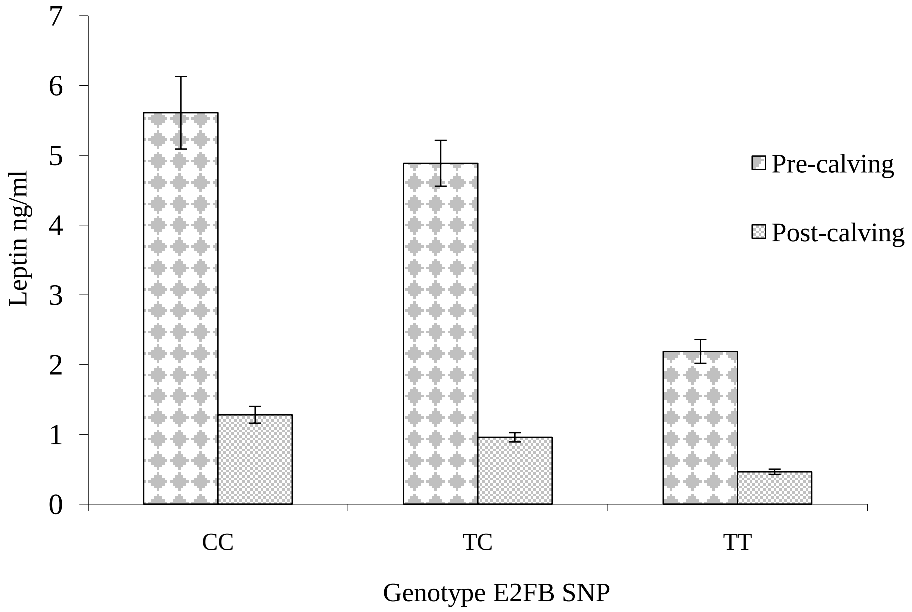


Figure 7-7: Mean pre- and post-calving leptin concentrations in VRC experimental animals with CC, TC or TT alleles of the E2FB SNP. All means differ significantly. Error bars represent standard errors.

7.6 Discussion

The reason for choosing the leptin gene as a focus of research is that leptin has a role as a lipostatic signal that regulates whole-body energy metabolism. Leptin is synthesised by white adipocytes (Zhang *et al.*, 1994; Chilliard *et al.*, 2001; Macajova *et al.*, 2004) and has a role in the regulation of appetite (Houseknecht *et al.*, 1998), reproductive performance (Hileman *et al.*, 2000) and food intake (Howie, 1999). It also affects body composition (Fitzsimmons *et al.*, 1998; Schenkel *et al.*, 2005). This makes leptin one of the best physiological candidate markers for liveweight, feed intake, energy expenditure, reproduction and certain immune system functions. The Australian cattle that were used in this experiment contain the genetic variants that have been described in the literature (Fitzsimmons *et al.*, 1998; Buchanan *et al.*, 2002; Nkrumah *et al.*, 2004b; Nkrumah *et al.*, 2005). The bovine leptin gene SNP UASMS1, UASMS2, E2FB and E2JW were mapped in all the cattle that were genotyped in this work. Identifying leptin SNPs to examine SNP/trait associations could be a useful tool in the development of MAS in beef cattle. In this discussion the following sections are considered:

- the frequency distribution of SNP alleles in an Australian experimental population of cattle;
- the frequency distribution of SNP alleles in an Australian experimental population of cattle compared to the allele frequency reported in Kononoff *et al.* (2005) and Nkrumah *et al.* (2005);
- the association between the SNP identified in our experimental cattle and carcass and efficiency traits;

- the association between SNP and pre- and post-calving blood parameter profiles.

7.6.1 The frequency distribution of SNP alleles in an Australian experimental population of cattle.

The Hardy–Weinberg Equilibrium (HWE) principle (Hardy, 1908) states that both allele and genotype frequencies in a population of animals remain constant and that they remain in equilibrium from generation to generation. Specific disturbing influences, such as other than purely random joining, are required to cause a shift away from this equilibrium.

The frequency distribution of SNP alleles in the Australian experimental cattle was compared to results from North American studies (Lagonigro *et al.*, 2003; Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005; Schenkel *et al.*, 2005). Table 7-9 compares the E2FB SNP in experimental cattle at VRC with the results obtained by Kononoff *et al.*, (2005). It shows that for the SNP frequency there is a significant difference between populations (P = 0.046, χ^2 = 6.15, df. = 2, see Table 7-3) and also a departure from the HWE. The numbers of animals in this analysis are large and it is likely that small differences in allele distribution between populations are likely to be statistically significant. The difference in the distribution of the alleles in the two populations is postulated to be due to the non-random joining, as well as the selection for a divergence in two specific traits in the Australian cattle. These same factors influence the departure from the HWE. Perfect HWE in nature is not possible as there are always influences that act to disrupt it in biological systems (Hardy, 1908; Emigh, 1980). The majority of animals in both populations are heterozygotes but there are differences in the homozygote percentages, specifically the CC genotype. It is concluded that the E2FB

SNP is found in Australian cattle but with a different distribution to that reported in previous studies, probably because of the specific nature of the genetic selection in the Australian cattle.

A factor that could have had an impact on this analysis was the limited number of sires represented in the experimental population. Within the Industry line there were multiple sires represented. However, within the NFI line there were far fewer sires represented because of the specific selective breeding programme over the last decade that has led to the production of a line of cattle with a marked divergence in NFI. It is possible that if "sire" were added to the statistical models as a random effect, significant differences between experimental populations might become less apparent. It was not possible to do this because of the absence of the relevant sire data.

Table 7-9: The frequency of the CC, CT and TT genotypes in E2FB SNP of the bovine leptin gene in cattle at VRC experimental cattle and in Kononoff *et al.*, 2005.

	VRC		Kononoff *et al.*, 2005.	
Genotype	no. of cattle	%	no. of cattle	%
CC	24	17.1	385	24.9
CT	71	50.7	780	50.5
TT	45	32.2	379	24.6
total	140	100	1544	100

Comparing the UASMS1 and UASMS2 SNP was done with results reported in Nkrumah *et al.* (2005). UASMS1 and UASMS3 are completely linked suggesting that for UASMS1 all allele frequencies and associations with traits can be extended to UASMS3 (Schenkel *et al.*, 2005). The linkage of UASMS1 and UASMS3 is reported in Nkrumah *et al.* (2005) but in that study they reported all allele frequencies and trait associations in relation to UASMS3. For the purpose of comparison, the UASMS1 results from VRC experimental animals have been compared to UASMS3 results reported by Nkrumah *et al.* (2005). In this report the genotype frequencies of the two SNPs were distributed according to HWE proportions in both populations ($P > 0.10$).

Table 7-10 compares the frequency distribution of UASMS1, UASMS2 and UASMS3 between VRC experimental cattle and those genotyped in the American study. For each SNP all genotypes are represented in both populations. It also shows that for the UASMS2 SNP frequency distribution there is a highly significant difference between populations (P = 0.001, x^2 = 14.35, df = 2) and therefore a departure from the HWE. Although not specifically identified in this work, it is likely that the mechanism behind this difference lies in the non-random joining and specific trait selection behind the breeding of the VRC experimental animals. For the UASMS1/3 SNP comparison there was no significant difference between the populations (P = 0.311, = x^2 = 2.34, df = 2). Again the significant conclusion is that the UASMS1 and UASMS3 SNPs are found in Australian cattle but possibly with a different distribution to that reported in previous studies, probably owing to the specific nature of the genetic selection in the Australian cattle.

Table 7-10: The frequency of SNP genotypes in UASMS1, UASMS2, and UASMS3 SNP of the bovine leptin gene in VRC experimental cattle and in a Nkrumah *et al.* (2005).

UASMS2					
VRC			Nkrumah *et al.*, 2005.		
Genotype	No. of cattle	%	Genotype	No. of cattle	%
CC	62	44	CC	99	66
CT	71	50	CT	45	30
TT	8	6	TT	6	4
total	141	100		150	100
UASMS1			**UASMS3**		
VRC			Nkrumah *et al.*, 2005.		
Genotype	No. of cattle	%	Genotype	No. of cattle	%
CC	26	18	CC	27	18
CT	76	53	CG	68	45
TT	41	29	GG	55	37
total	143	100		150	100

Schenkel *et al.* (2005), when reporting allele frequency for the E2JW SNP in a Canadian population of cattle, indicated that this SNP showed the largest difference compared to the other SNPs discussed in this chapter. The T allele was rare compared to the A allele (4.0 vs. 96%) and these results are consistent with those reported by Lagonigro *et al.* (2003) as well as with the results from the VRC experimental cattle.

The VRC experimental animals were classified according to experimental genotype (see Table 7-1) and the allele frequency distribution was analysed. The results of a Pearson Chi-Squared test are shown in Table 7-3. The reason for the difference in the allele frequency and genotype distribution of E2FB and E2JW SNP has not been identified but it is highly likely to be due to the non-random joinings that took place in this experiment. For the E2JW SNP only NFI animals were genotyped and these animals in particular were specifically joined to bulls with EBVs for either high- or low-NFI and therefore experimental genotypes do not share sires at all. This departure from the HWE is also described in Schenkel *et al.* (2005) and in Nkrumah *et al.* (2005) where animals in those reports were from different genetic lines or different breeds, but the overall frequency and distribution of alleles is the same. The only other Australian study that addresses the SNP (Barendse *et al.*, 2005) also found a genotype frequency distribution similar to that found in the North American studies.

Contrary to our first hypothesis, the frequency distribution of the four SNP genotyped in VRC experimental cattle was not similar to that described in studies of North American populations of cattle. The difference in allele/genotype frequency and distributions between the populations of cattle is not unexpected, especially considering the specific selection of particular experimental Genotypes in the VRC experimental cattle. The notable finding is that all genotypes of all SNPs exist in both populations of cattle, which prompts an investigation in Australian cattle of the SNP/trait associations documented in North American papers.

7.6.2 The association between the SNP identified in VRC experimental cattle and carcass and efficiency traits.

There were few SNP/trait associations identified in the Australian experimental population used in this work, whereas North American studies report numerous SNP/trait associations between SNPs and fatness, lean meat yield, EMA, marbling, growth, ultrasound measures of back fat, feed intake and NFI (Fitzsimmons *et al.*, 1998; Buchanan *et al.*, 2002; Liefers *et al.*, 2002; Liefers *et al.*, 2003a; Crews *et al.*, 2004; Nkrumah *et al.*, 2004b; Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005; Schenkel *et al.*, 2005; Lusk, 2007)

Table 7-4 shows the SNP/trait associations examined in this experiment and shows that the only significant association was between the E2JW SNP and NFI and post-test ultrasound P8 fat measures. It was found that animals with an A allele, particularly homozygotes, have significantly less P8 fat measured on ultrasound, whereas Schenkel *et al.* (2005) reported the opposite, viz that the it was the T allele that was associated with higher lean meat yields and lower measures of fatness. E2JW SNP/trait associations have not previously been examined in Australian cattle.

The absence of any other SNP/trait association concurs with the findings of Barendse *et al.*(2005), who examined similar SNP/fatness trait associations for the E2FB SNP in a large number (3129) of cattle and found no association with several fatness traits. It is not possible to draw firm conclusions pertaining to associations between SNP and carcass traits from the current experiment because of the small number of animals used. However, the results generally support the conclusion reached by Barendse *et al.*(2005) that the leptin SNPs are unlikely to be of genetic importance in Australian cattle in terms of associations with body composition. It is possible, though, that because the Barendse *et al.*(2005) study used young animals, associations with fatness may not have become evident as animals had not finished growing and depositing fat. In the VRC experimental animals, although there was no SNP/trait associations when the animals were measured pre and post-calving, the animals were all female and still only in their first parity. Any trait associations in males, or in fully mature cows, could not be determined. The heterogeneity of cattle used within and between all the studies in this field, in terms of age, diet, sex and breed, is likely to be the source of the differing findings that studies have reported previously, and our findings are not an exception.

The association between the E2JW SNP and NFI EBV (see Figure 7-4) identified in the data set which included extra Trangie Research Station sourced animals, whereby animals with the T allele had higher NFI EBVs, has not been reported before. Lagonigro *et al.* (2003) reported that average feed intake of bull calves from 6 to 12 months was higher if they had the AT genotype than the AA genotype but there was no reference to NFI. Where others (Crews *et al.*, 2004; Nkrumah *et al.*, 2004b; Nkrumah *et al.*, 2005) have reported associations between SNP and NFI, in particular for the UASMS2 and E2FB SNP, no such associations were found in the results from this experiment. However, the pattern of shown by homozygous AA animals recording lower NFI values than heterozygotes or homozygous TT animals documented for the E2FB and UASMS2 SNP was the same as the E2JW SNP analysed in this experiment.

The occurrence of significant associations between SNP and feed efficiency is interesting in that if a marker for feed efficiency could be validated across extensive populations and breeds, it would greatly assist in the selection for this desirable trait which is otherwise expensive and time consuming to measure. The difficulty lies in the fact that the SNP/trait relationships were not consistent between populations and when they are investigated further, the ability to use the markers to select for the desirable trait (low-NFI) becomes much reduced. Table 7-6 shows the proportion of animals per SNP genotype falling into the arbitrary category of high-NFI (EBV > 0.5), average- (EBV – 0.5 – 0.5) and low-NFI (EBV < - 0.5). This arbitrary classification was used because it mirrors the range of NFI EBVs within each experimental genotype (high-NFI or low-NFI) in the VRC experimental animals. Figure 7-5 depicts that despite the significant association between the AA genotype and lower NFI, selection for increased feed efficiency based purely on SNP genotype alone would result in 58% of the animals being within either the average- or the high-NFI category. This highlights the fact that the leptin SNPs are not appropriate candidates for MAS, where the target trait is improving feed efficiency, and thus the measurement and evaluation of this trait in beef cattle remains expensive and time consuming.

The results from this experiment identify the potential to associate SNPs in the bovine leptin promoter and exon regions which would assist MAS, but the results are not consistent with those other studies and need to be validated across a larger population, of particularly Australian cattle, of varying ages and sex. This conclusion reflects the difficulty of investigating SNP/trait associations in general, as often associations present in one population are not present in another, and the numbers required to validate significant findings are often large.

7.6.3 The association between SNP and pre- and post-calving blood parameters

Given the importance of energy balance in the efficiency and productivity of a beef herd, it was useful to investigate the association between leptin gene SNP and various indicators or regulators of physiology and in particular fat metabolism. These included leptin, IGF-1, GH and insulin. If it were to be shown that the SNP were on the whole associated with a particular concentration of these endocrine factors, it could lead to MAS whereby animals with a predisposition to greater feed efficiency or leanness would be easily identified in populations through genetic analysis. An extensive investigation into these association has not been done, but, Nkrumah *et al.* (2005) did report that the T allele of UASMS2 was significantly associated with serum leptin concentrations ($P < 0.001$) and showed significant additive and dominance effects. Animals with the TT genotype had significantly ($P < 0.001$) higher circulating leptin concentrations than both the CT and CC animals. They reported no association with any other endocrine factor. Buchanan *et al.* (2002) found that when analysing the E2FB SNP in the bovine leptin gene, animals with the T allele expressed higher levels of leptin mRNA than those with the A allele, a result similar to the one in the current experiment. This suggested that the E2FB SNP was a causative mutation. Only these researchers have postulated a molecular and physiological mechanism for the increased expression of leptin mRNA. They hypothesised that an increase in leptin expression could be a feedback response in compensation for reduced biological function of the hormone itself. Other researchers have reported a similar biological effect of an arginine amino acid substitution (Inaba *et al.*, 2001; Ribba *et al.*, 2001). Buchanan *et al.* (2002) hypothesised that the amino acid change from arginine to cysteine was imparting a functional difference to the leptin molecule and suggested that through a shape change the cysteine's presence in the A-helix of the leptin molecule may disrupt the binding of leptin to its receptor. Another explanation for the functional change was that the presence of another unpaired cysteine in the leptin molecule could destabilise the disulfide bridge found between the existing cysteines which has been shown to be critical for biological function (Zhang *et al.*, 1994; Rock *et al.*, 1996).

Table 7-8 shows the significant associations between SNP and endocrine measures pre- and post-calving in the VRC experimental animals and shows that the only significant relationships that exist are between the UASMS1 and E2FB SNP and pre- and post-calving leptin concentrations. Although the SNP identified by Nkrumah *et al.* (2005) was not significant in this experiment, the pattern of significance was the same. Animals carrying the T allele had significantly higher pre- and post-calving leptin concentrations than the homozygous AA animals, and the homozygous TT animals had higher leptin concentrations than the TC animals (see Figure 7-6 and Figure 7-7). Pre-calving concentrations were uniformly higher than post-calving. It has been shown that serum leptin is positively associated with liveweight and body fatness (Chilliard *et al.*, 1998b; Geary *et al.*, 2003; Liefers *et al.*, 2003a). In this experiment the UASMS1 and E2FB SNPs were not associated with body fatness and it is therefore concluded that these SNPs do not represent functional mutations in the leptin gene. However, in the

Nkrumah *et al.* (2005) report the UASMS2 SNP was found to be strongly associated with both leptin concentrations and body fatness and it was concluded that this SNP was indeed functionally significant. Nkrumah *et al.* (2005), who originally identified the SNPs in the promoter region of the leptin gene suggested that the exact molecular and physiological mechanisms underlying the association of the polymorphisms with traits reported in their study are unknown. They suggested that to identify the possible functionality of the promoter variants, *in vivo* and *in vitro* experiments would be required. They speculated that the location of the present SNPs, especially UASMS2, in the regulatory region of the leptin gene makes them potential regulators of leptin expression in cattle, and possibly serve as surrogates for causative SNPs that are yet to be detected. The physiological mechanisms behind the SNP/trait associations in the results presented in this thesis were not investigated in this experiment, but the author concurs that experiments to further elucidate molecular mechanisms behind SNP effects are necessary and may identify further functionally significant SNPs.

7.7 Conclusions

The results of the current experiment suggest that identifying leptin gene SNP in Australian cattle is unlikely to be a useful tool in the development of MAS, particularly when considering the desirable heritable traits NFI and leanness. Although the SNPs that have been reported in North American cattle are present in Australian cattle, the distribution of SNP genotypes is not the same and is postulated to be different if a larger population of animals were to be examined. Although there were some SNP/trait associations with carcass traits, they were not the same as those previously reported and probably of little industry relevance. It is likely that the absence of any major SNP/trait associations is due to the limited number of animals used in the current experiment and although the possibility of using the SNP for MAS is still worth considering, this experiment provides no evidence supporting this hypothesis. The experiment was a useful first step in the examination of the leptin gene SNPs in Australian cattle and their association with carcass and efficiency traits, but further research is required to validate the findings of the current experiment.

CHAPTER 8. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

This thesis has addressed the impact of genetic selection for the desirable traits leanness and feed efficiency on the female beef herd. The aims were to consider the concerns raised by the beef producing industry about single trait selection over time and its possible detrimental effect on measures of productivity in the female herd. These measures were encompassed under the term MP, and the experiments were designed to scientifically evaluate the impact of selection for either leanness or feed efficiency on MP, and give producers more knowledge about how these traits will affect the breeding herd.

The experiments were designed to measure and quantify the amount of fat storage and retrieval, and predict carcass yield in the experimental animals over two breeding cycles and under two (high- and low-) nutritional treatments. The main findings were that Fat and high-NFI animals were fatter, and had lower yield predictions than Lean and low-NFI animals throughout the year and on both nutritional treatments. This result concurs with much of the published literature and supports three main conclusions. The first is that the selection for a divergence in fatness is possible using EBVs and that this difference persists throughout the breeding life of the animal. Also, fatness differences between high and low feed-efficient animals, previously documented in young, grain-fed animals, persist in the female herd after two parities. Thirdly, when using a published equation that considers several ultrasound carcass measures and liveweight, it was possible to detect differences in predicted carcass yield between animals and it was confirmed that an additional economic benefit of selection for leanness or feed efficiency was that these animals had higher predicted carcass yields.

This experiment ended for the author after two parities but continues at the two sites. It would be useful to analyse fat distribution in the experimental animals at the end of their breeding life. Differences between Genotypes at this time may reflect differences in maturity pattern that may possibly contribute to breeding longevity, a key component of MP that could not be determined in the current experiment. It would also be useful to measure yield in the slaughtered animal and compare this result with the results of the yield prediction analysis.

After calving, return to a physiological state that is favourable for conception and maintenance of a pregnancy is a very important contributor to MP. In a profitable beef herd this needs to happen within a certain time because of the seasonal calving pattern used by most Southern beef producers. Measuring the PPAI is one way to quantify this trait. Another is using DTC, which is the interval from mating-start-date to the calving date of an individual cow. Both of these measures were used to identify any differences in PPAI in the different Genotypes.

Fortnightly assays of serum progesterone were used to determine when an animal had ovulated after calving. A significant increase above a baseline concentration for each animal was used to signify a prior ovulation. No differences in PPAI between Genotype were identified using this method but animals that calved later in the season ovulated sooner than the early-calving animals. It was postulated that the greater availability of feed in the later parts of the calving season was the reason for this, highlighting the important link between adequate nutrition and return to oestrus post-partum.

Measuring progesterone fortnightly was too infrequent to gain the resolution necessary to discriminate PPAI between Genotype or treatments. Physical methods, such as the use of Estrus Alerts®, were also not useful in this process because they either rubbed off in wet and muddy conditions, or were too difficult to interpret. Ideally, progesterone would be measured at least twice a week. This would identify the progesterone concentrations in animals at all stages of the oestrous cycle and animals at the start or end of the cycle would still be identified as post-ovulatory whereas they were probably missed using the current methodology. It would also have been very beneficial to have an ultrasound evaluation of ovarian activity at least twice a week. Animals that had recently ovulated could have been identified by the presence of a CL. Unfortunately the financial and logistical difficulties associated with the increased handling of the cattle and extra assays precluded the researchers from conducting the more intensive study suggested here.

In future, a scaled-down version of the current experiment to specifically address the shortfalls in the measurement of PPAI in animals of different Genotypes is indicated. Post-partum return to oestrus is a vital contributor to MP and the conclusions of the current experiment would have benefited from this information. Fewer animals could be used with daily ovarian scans and bi-weekly progesterone measures taken to properly elucidate the extent to which selection for leanness or feed efficiency impacts on PPAI.

As hypothesised, nutrition rather than Genotype impacted on production and efficiency parameters in the experimental animals. On low-nutrition cows had longer measures of DTC than those on high-nutrition. Cows had calves that grew more slowly and weaned lighter. Animals on low-nutrition ate fewer MJ ME per kg beef weaned yet there were no differences between experimental Genotypes in any of these parameters. The most significant and industry-relevant finding in this experiment was the trend suggesting that not only do low-NFI animals eat less than their high-NFI counterparts when grazing green pasture, but they also wean more beef for every MJ ME they consume. This, rather than confirming producers' fears about the negative impact of selection for feed efficiency on productivity, suggests the opposite. More highly feed-efficient animals do in fact eat less feed and MP was not compromised, even on a plane of low-nutrition, suggesting that the economic benefits of selection for this desirable trait persist in energy-restricted, grazing animals.

A few things need to be done to validate the result that suggests low-NFI cattle eat fewer MJ ME per kg beef weaned. Difficulties in the estimation of pasture intake in replicate groups led to a high degree of variation between replicates and probably contributed to the lack of statistical significance of the result. Increasing the number of replicate groups and the accuracy of the data collection will possibly solve this problem. The VRC experiment is currently being replicated in South Australia where there are three, instead of just the two replicate groups. That experiment uses small grazing cells and cattle are moved every two days so estimation of pasture disappearance will probably be more accurate. Combining the results of that experiment with the current experiment, and with data from an additional two breeding cycles, will overcome the problems encountered thus far by the author.

Conclusions about the impact of restricted nutrition on productivity and efficiency in the experimental Genotypes need to be tempered with the knowledge that the nutritional restriction was not extreme. This, as outlined in the thesis, was because of the constraints of treating all replicate groups equally in terms of nutrition and having a trigger for nutritional intervention being when one animal fell below BCS 1.5. The outcome was that the animals on the low-nutrition treatment were not, on average, extremely thin. However, conditions in the experiment do closely replicate the conditions experienced in a Southern grazing enterprise during times of energy deficit, such as drought or the autumn feed gap. The intervention point here was similar to the intervention point in a well-run commercial beef herd because farmers, on the whole, have an understanding of the importance of body condition when it comes to reproduction and overall MP. An experiment that tested Genotype differences under extreme nutritional restriction would be interesting and it is hypothesised that Genotype differences may be more pronounced under these circumstances, but this was not the point of the current research.

The trends discussed above are a positive result for the beef producing industry, not only in relation to the NFI Genotype but also for the Industry line where the trend towards leaner cattle being more efficient existed but was less statistically significant. Establishing that leaner or more feed-efficient cows eat less and produce more beef for what they eat, despite restricted nutrition and without compromising fertility, will mean producers can confidently choose to select for these traits if they suit the producers' enterprise targets.

A final aim in this thesis was to establish whether a physiological or genetic marker existed that would distinguish one Genotype from another. To do this various blood parameters were measured post-calving, when the female's physiology is most susceptible to negative energy balance. Analysis of the distribution and significance of Bovine Leptin Gene SNPs was also carried out to look for tools that could be used in marker-assisted-selection.

Blood parameter analysis revealed that the blood parameters, both endocrine and biochemical metabolites, were related in order of importance to body condition, energy balance and Genotype, but only when the difference between Genotypes mirrored a difference in body condition. This result supports the major hypothesis of this chapter. BHB, leptin, IGF-1 and acetate were all on the whole reflective of adiposity or nutritional treatment, consistent with the physiological responses discussed in other literature.

No single parameter acted as a marker for a particular Genotype. The physiology of cows in any one Genotype was not particularly unusual or unexpected, meaning that the recognised impacts of fatness and negative energy balance on reproduction can be considered applicable to all the experimental cattle. No hormone or biochemical pathway was more or less severely impacted upon by energy restriction of selection for a particular desirable trait. Elucidation of more complex endocrine pathways, such as the adrenalin sensitivity of the muscle and insulin responsiveness in animals of different Genotypes, would be an avenue for future research. Techniques such as insulin-clamps and adrenalin sensitivity experiments could be conducted on a small number of conditioned animals for each Genotype to determine if there are differences in the homeorhetic shifts that occur in animals during pregnancy.

Lastly, this thesis addressed the possibility of using SNPs in the bovine leptin gene as markers for feed efficiency or other carcass traits. The results suggested that although SNPs identified in other studies do exist in the experimental animals, the distribution of these SNPs is different to that reported in other literature and the marker/trait associations were not strong. It is postulated that is unlikely that a single mutation will have a large significant effect on a polygenic trait such as fatness or feed efficiency. The leptin gene was, however, a good candidate gene for MAS because of the association of leptin with fatness, but in the current experiment the numbers of animals tested were too few to reach robust conclusions about MAS using the leptin gene. It would be worthwhile to genotype several thousand Australian animals and it is possible that the weak marker/trait association identified in this work may be strengthened by the extra data.

This thesis has investigated the impact of genetic selection for feed efficiency and leanness on the female herd using animals selected for a divergence in each trait and subjected to two nutritional treatments. It has examined fat distribution, measures of fertility, an evaluation of herd efficiency, and differences in animal physiology and genetic makeup. There was no strong evidence to suggest that selection for the experimental traits compromised MP after two breeding cycles. This is an encouraging finding for beef producers. This conclusion needs to be tempered with the knowledge that the experiment is continuing for several more generations and the conclusions might change. The author looks forward to continuing his involvement in this exciting research.

CHAPTER 9. APPENDICES

9.1 Timeline of important periods during the experiment at Vasse Research Centre

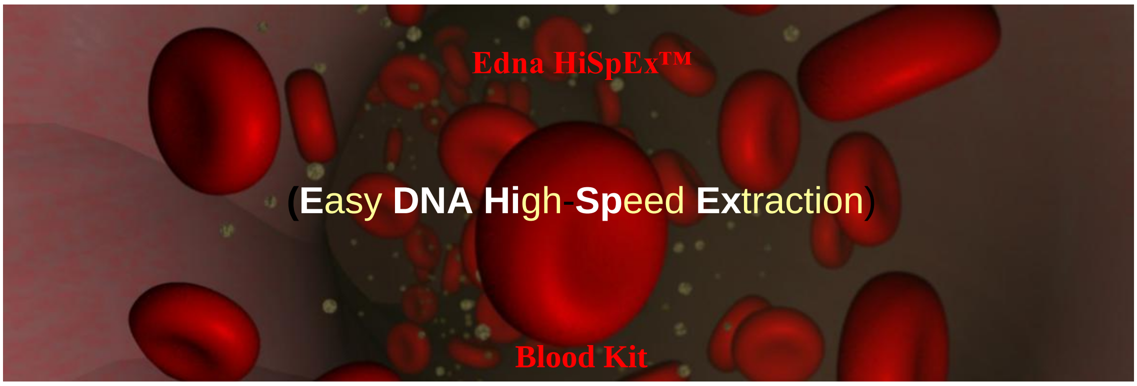
2006			
January			
February			
March	1st cohort NFI cattle arrive		
April	NFI feed-test 1st cohort	1st cohort industry cattle arrive	
May			
June			
July			
August		accredited scan 1st cohort	
September	joining 1st cohort		
October			
November			
December			
2007			
January	supplementary feeding	2nd cohort industry cattle arrive	accredited scan 1st cohort
February		pregnancy testing	
March			
April		2nd cohort NFI cattle arrive	
May		allocation of 1st cohort to experiment	pasture measurements
June	calving 1st cohort	NFI feed test 2nd cohort	
July			
August		accredited scan 1st and 2nd cohort	
September	joining 1st and 2nd cohort		
October			
November			
December			

2008

	supplementary feeding	accredited scan 1st and 2nd cohort	
January			
February		pregnancy testing	
		allocation of 2nd cohort to experiment	
March			
April			
May			pasture measurements
June	calving 1st and 2nd cohort		
July			
August			
September	joining 1st and 2nd cohort		
October			
November			
December			

9.2 Edna HiSpEx™ Blood Kit

100 Extractions



Edna HiSpEx™

produces denatured DNA* suitable for PCR and related processes. It has been designed for extraction of DNA using manual or high-throughput robotic systems and does not require centrifugation.

Edna HiSpEx™ comes in kit form for blood, animal tissue and plant tissue.

Edna HiSpEx™ Blood Kit has been validated for extraction of PCR-ready DNA from EDTA and citrate preserved blood. The use of 1µl of extract as template in a 10µl PCR reaction is suggested as a starting point. Some PCR's may require optimisation of extract volume used.

Blood Extraction Method

Note: This process is suitable for whole or spun, chilled or frozen, fresh, EDTA or Citrate preserved blood.

Add 5µl of blood to 20µl of Solution 1. Mix well.

Add 5µl of this mixture to 16µl of Solution 2. Mix well.

Add 59µl of Solution 1 to the mixture in step 2. Mix well. The extract solution should be homogeneous with no cell clumps.

Incubate at 95°C for 15 minutes.

Add 20µl of solution 3. Mix well. If precipitates form, these should be avoided in PCR, however their presence will not damage the extract.

The DNA is now ready for PCR analysis or storage (-20°C).

For research purposes only.

For *in vitro* use only. Avoid contact with skin, eyes or other sensitive areas.

Store Kit at 2-10^{0}C

PCR products generated from Heat Shock Protein Universal primers on DNA template extracted using Edna HiSpEx Blood lot #006B (Soln. 1, A75B; Soln. 2, 49B; Soln 3, 102B).

Lane 1: DNA marker (pCU19/ HpaII, 0.5µg)

Lane 2: Negative control (water)

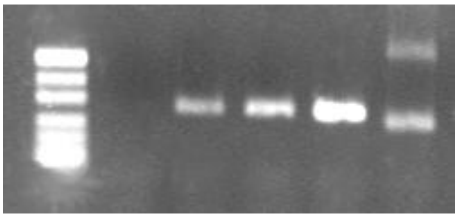
Lane 3: Human Blood (on FTA card)

Lane 4: Human blood (frozen, EDTA)

Lane 5: Cattle blood (stored at 4°C for

>1.5yo)

Lane 6: Ostrich blood (fresh)



9.3 NFI animals – Angus ID, sire ID and date of birth

Angus ID	Sire ID	Date of Birth	Cohort
NDAA211	T024	21/07/05	2005
NDAA200	U051	20/07/05	2005
NDAA151	U005	17/07/05	2005
NDAA328	T024	10/08/05	2005
NDAA304	U116	07/08/05	2005
NDAA215	U077	21/07/05	2005
NDAA318	T024	09/08/05	2005
NDAA306	U005	07/08/05	2005
NDAA326	T024	10/08/05	2005
NDAA329	T024	11/08/05	2005
NDAA294	U116	05/08/05	2005
NDAA354	U077	15/08/05	2005
NDAA142	U015	16/07/05	2005
NDAA426	NEPW102	02/09/05	2005
NDAA126	U077	15/07/05	2005
NDAA307	T024	07/08/05	2005
NDAA048	T034	09/07/05	2005
NDAA085	U005	13/07/05	2005
NDAA187	U077	19/07/05	2005
NDAA077	U005	12/07/05	2005
NDAA044	T024	08/07/05	2005
NDAA120	U077	15/07/05	2005
NDAA116	U005	14/07/05	2005
NDAA065	T024	11/07/05	2005
NDAA309	U005	08/08/05	2005
NDAA091	U015	13/07/05	2005
NDAA184	T034	19/07/05	2005
NDAA052	U077	10/07/05	2005
NDAA094	U077	13/07/05	2005
NDAA409	NEPW102	30/08/05	2005
NDAA349	T119	14/08/05	2005
NDAA358	U227	16/08/05	2005
NDAA110	T119	14/07/05	2005
NDAA458	NBBW118	08/09/05	2005
NDAA372	NBBW118	18/08/05	2005
NDAA255	U124	27/07/05	2005
NDAA113	U040	14/07/05	2005
NDAA270	U124	31/07/05	2005
NDAA331	U040	11/08/05	2005
NDAA057	NBBW118	10/07/05	2005
NDAA188	T119	19/07/05	2005
NDAA119	T095	14/07/05	2005
NDAA321	U124	10/08/05	2005
NDAA341	NBBW118	13/08/05	2005
NDAA289	U090	04/08/05	2005
NDAA273	T119	31/07/05	2005
NDAA298	T119	06/08/05	2005

NDAA330	U090	11/08/05	2005
NDAA286	U123	03/08/05	2005
NDAA165	U040	19/07/05	2005
NDAA214	U124	21/07/05	2005
NDAA368	T095	17/08/05	2005
NDAA291	U090	05/08/05	2005
NDAA152	T119	17/07/05	2005
NDAA283	U090	02/08/05	2005
NDAA104	T095	13/07/05	2005
NDAA415	NEPW102	31/08/05	2005
NDAA251	NBBW118	27/07/05	2005
NDAA443	NEPW102	06/09/05	2005
NDAA385	NEPW102	24/08/05	2005
NDAB322	U005	05/08/06	2006
NDAB454	T024	21/08/06	2006
NDAB156	T024	26/07/06	2006
NDAB123	U005	23/07/06	2006
NDAB529	Y025	08/09/06	2006
NDAB379	T024	10/08/06	2006
NDAB455	U051	21/08/06	2006
NDAB147	U077	26/07/06	2006
NDAB366	T024	08/08/06	2006
NDAB225	U077	30/07/06	2006
NDAB197	U005	28/07/06	2006
NDAB174	T024	27/07/06	2006
NDAB148	U124	26/07/06	2006
NDAB195	T024	28/07/06	2006
NDAB286	U077	02/08/06	2006
NDAB218	T024	29/07/06	2006
NDAB396	U227	13/08/06	2006
NDAB258	T024	31/07/06	2006
NDAB308	U124	03/08/06	2006
NDAB414	U051	14/08/06	2006
NDAB304	U005	03/08/06	2006
NDAB487	T034	24/08/06	2006
NDAB494	T024	25/08/06	2006
NDAB227	T119	30/07/06	2006
NDAB465	S472	22/08/06	2006
NDAB172	T119	27/07/06	2006
NDAB117	T119	22/07/06	2006
NDAB135	U124	25/07/06	2006
NDAB203	T024	28/07/06	2006
NDAB367	U005	08/08/06	2006
NDAB058	Z008	10/07/06	2006
NDAB502	S472	26/08/06	2006
NDAB460	S472	22/08/06	2006
NDAB486	T095	24/08/06	2006
NDAB281	T119	02/08/06	2006
NDAB153	T119	26/07/06	2006
NDAB360	U124	07/08/06	2006
NDAB208	U040	29/07/06	2006

NDAB470	S472	23/08/06	2006
NDAB184	T119	28/07/06	2006

9.4 Industry animals EBVs

Angus ID	Sire ID	Date of Birth	Producer	Mid Parent rib fat EBV	Mid Parent MCW EBV	Mid Parent P8 EBV	Rib Fat EBV Feb 2007	Rib Fat EBV Jan 08
WMYA5	USAN088	07/03/05	MacLeay	-1.5	76.1	-2	-1.4	
WMYA47	USAN088	16/03/05	MacLeay	-1.2	86.5	-1.8	-1.3	
WMYA56	NZE848	17/03/05	MacLeay	1.5	47.7	2	2.4	
WFNA39	NGMT30	26/03/05	Coffey	1.6	64.1	2.1	1	
WFNA44	NGMT30	27/03/05	Coffey	1.2	40	1.6	0.8	
WFNA55	WFNW008	28/03/05	Coffey	1.2	67.9	1.4	3.4	
WMYA145	WMYY201	28/03/05	MacLeay	-1.2	60.8	-1.5	-1.1	
WMYA153	WMYY6	29/03/05	MacLeay	-0.9	80.2	-1.3	-1.8	
WFNA64	WFNW008	30/03/05	Coffey	1.3	68.7	1.7	3.2	
WFNA61	USA315	30/03/05	Coffey	0.8	52.7	0.6	0.6	
WMYA157	WMYY201	30/03/05	MacLeay	-0.9	71.9	-1.4	-1.9	
WFNA75	VTMM126	02/04/05	Coffey	2.6	42.4	1.5	0.9	
WFNA76	VTMM126	02/04/05	Coffey	1			1.3	
WFNA72	WFNW008	03/04/05	Coffey	1.3	66.8	1.8	2.4	
WFNA73	WFNW008	03/04/05	Coffey	0.8	68.3	0.9	1.2	
WATA40	VLYV329	03/04/05	Kuss	-1.2	79.4	-1.9	-1.4	
WFNA95	NGMT30	05/04/05	Coffey	0.9	52.6	1	1	
WFNA96	NGMT30	05/04/05	Coffey	0.9	53.6	1	1.4	
WFNA98	WFNW008	06/04/05	Coffey	1.3	62.8	1.7	0.8	
WJYA141	QBGV50	08/04/05	Young	-1.35	83		-1.9	
WBPA7	WBPX197	10/04/05	Hockey	-0.9	92.8	-1.4	-0.8	
WBPA177	WBPX197	10/04/05	Hockey	-1.2	98.3	-1.9	-1.7	
WFNA139	USA3130	11/04/05	Coffey	1.5	73.8	1.8	0.8	
WFNA138	USA3130	11/04/05	Coffey	1.5	70	1.7	1.6	
WFNA136	WFNW008	11/04/05	Coffey	2.1	59.9	3	2.2	
WMYA196	WMYY163	11/04/05	MacLeay	-1.2	65.7	-1.6	-1.5	
WATA76	WKHW31	12/04/05	Kuss	-1.4	97.7	-1.8	-1.3	
WMYA199	WMYY201	12/04/05	MacLeay	-0.9	69.5	-1.2	-1	
WMYA197	WMYY201	12/04/05	MacLeay	-1.1	57.3	-1.3	-1.1	
WBPA205	WBPX22	13/04/05	Hockey	-1.8	89.4	-2.5	-2.4	
WMYA201	WMYY163	13/04/05	MacLeay	-1.1	93.8	-1.7	-2	
WFNA151	VTMM126	14/04/05	Coffey	0.8	46.4	1	1.4	
WMYA203	WMYY26	14/04/05	MacLeay	-0.9	88.9	-1.2	-1.5	
WJYA133	QBGV50	17/04/05	Young	-1.5	81.5		-1.6	
WBPA9	WBPW242	20/04/05	Hockey	0.8	48.2	0.9	0.6	
WFNA192	WFNX31	21/04/05	Coffey	1.3	48.9	1.5	0.4	
WFNA188	WFNW008	21/04/05	Coffey	1.4	60.5	1.8	0.7	
WATA88	WDCX48	22/04/05	Kuss	-0.9	78.1	-1.2	-1.8	
WATA91	WDCX48	24/04/05	Kuss	-1.2	71.1	-1.5	-1.5	
WATA97	WKHW133	28/04/05	Kuss	-1.3	92.7	-1.8	-1.8	
WJMA47	NGMW245	28/04/05	McGregor	-1.8	98	-2.5	-2.1	

WJMA259	WJMW121	07/05/05	McGregor	1.3	66.7	1.7	1.6	
WJMA24	USA1299	07/05/05	McGregor	1.1	79.8	1.2	1.6	
WFNA228	WFNW008	09/05/05	Coffey	1.2	57.6	1.5	2.7	
WBPA118	WBPX22	13/05/05	Hockey	-0.9	86.6	-1.3	-1.8	
WMYA240	WMYY26	22/05/05	MacLeay	-1.5	93.9	-2.1	-1.9	
WFNA252	WFNW008	25/05/05	Coffey	1.6	67.1	2	2.9	
WJMA91	WJMX17	26/05/05	McGregor	0.9	77.7	1	1.6	
WJMA342	WJMX18	29/05/05	McGregor	0.9	54.2	1	1.2	
WBPA188	WBPX22	31/05/05	Hockey	-1.4	79.5	-1.9	-1.3	
WFNA256	WFNW008	01/06/05	Coffey	0.9	77.9	1	0.2	
WJYA113	NBBX79	01/06/05	Young	-1.5	70		-1.3	
WJMA364	WJMX18	03/06/05	McGregor	1	36.6	1.2	2.3	
WBPA101	WBPX22	07/06/05	Hockey	-1.4	72.8	-1.9	-1.1	
WBPA93	WBPT66	10/06/05	Hockey	-1.7	68.9	-2.5	-2.2	
WJYA90	QBGV50	16/06/05	Young	-1.9	85.5		-1.6	
WBPA85	WBPW251	19/06/05	Hockey	-1.2	58.3	-2	-1.4	
WJYA145	WJYX30	25/06/05	Young	0.95	64		0.5	
WJMA186	WJMW1	11/07/05	McGregor	-1.8	90.4	-2.7	-1.2	
WJMA192	WJMW1	17/07/05	McGregor	0.8	56.5	0.7	0.8	
WMYB2	VVXV536	28/02/06	1.3	70.9	0.9			0.9
WMYB16	NGMT30	10/03/06	1.7	70.5	2.2			2
WMYB25	VVXV536	12/03/06	1.1	79.6	0.7			1.5
WMYB47	WMYZ63	15/03/06	1.9	39.2	2.3			1.4
WMYB44	NZE98787	15/03/06	-1.1	86.6	-1.6			-0.5
WMYB61	NZE536	16/03/06	-1.6	95.2	-2.1			-1.5
WFNB23	WFNZ54	17/03/06	1.4	76.5	1.6			1.1
WMYB76	VVXV536	18/03/06	1.1	68	0.6			0.7
WFNB27	NGMW391	18/03/06	-1.1	56.9	-1.6			-1.2
WMYB80	USA0713	18/03/06	-1	57.9	-1.4			-0.4
WMYB125	VVXV536	24/03/06	1.3	52.2	0.7			1.3
WMYB122	NGMT30	24/03/06	1.4	66.3	1.8			1.3
WMYB133	NZE0122	25/03/06	1.2	55	1.6			1.1
WMYB145	WMYZ156	26/03/06	-1.4	73.3	-1.9			-0.6
WMYB154	WMYZ63	27/03/06	0.8	50.5	1.1			1
WFNB64	USA475842	29/03/06	0.8	61.4	0.9			1.3
WFNB112	WFNX33	04/04/06	-1.1	36.5	-1.5			-0.6
WFNB120	NAQX15	05/04/06	-1.3	51.4	-1.9			-1
WFNB149	WFNZ54	10/04/06	0.8	61	0.9			0.6
WMYB205	WMYZ63	10/04/06	0.9	58.8	1.2			0.7
WMYB207	WMYZ53	11/04/06	-0.8	99.9	-1			-0.6
WFNB157	WFNZ54	12/04/06	1	64.2	1.1			0.5
WMYB210	WMYZ163	12/04/06	-0.9	87	-1.3			-0.8
WATB10	NORY283	14/04/06	1	67	1			0.7
WATB12	NHZY1282	14/04/06	0.8	66	0.9			0.6
WFNB197	WFNZ12	18/04/06	-1.2	62.9	-1.8			-0.3
WJYB108	WHHY24	20/04/06						-1.2
WFNB207	WFNX33	21/04/06	-0.8	60.4	-1.2			-0.4
WMYB231	WMYZ53	22/04/06	-0.8	76.8	-1.1			-0.5
WJYB118	NBBX79	25/04/06						-1.3
WATB61	NORY283	26/04/06	0.8	61.7	0.8			1.1
WJMB36	WJMY18	01/05/06	2.1	66.7	2.5			1.6

WJMB43	PROUDRAM B43	04/05/06	-1	83.9	-1.5	-0.3
WMYB254	WMYZ156	07/05/06	-0.9	84.1	-1.2	-1.1
WJMB254	WJMY18	11/05/06	1.2	65.3	1.2	0.6
WFNB266	WFNZ87	13/05/06	-1	74.4	-1.6	-1.1
WATB100	WKHW31	19/05/06	1.2	73.3	1.7	1.5
WFNB281	WFNZ87	01/06/06	-0.8	69.2	-1.3	-0.8
WATB115	WKHW133	17/06/06	-1	87.7	-1.6	-1.2
WJYB201	NBBX79	13/07/06				-1.1

9.5 NFI animals EBVs

Tag	Midparent NFI EBV	Updated NFI EBV post test	Adjusted actual NFI post test	NFI EBV at allocation
NDAA289	-0.69	-0.98	-1.43	-0.98
NDAA291	-0.69	-0.77	-0.75	-0.77
NDAA306	0.68	0.53	0.57	0.53
NDAA211	0.68	0.73	0.9	0.73
NDAA126	0.71	0.56	0.28	0.56
NDAA349	-0.61	-0.56	-0.21	-0.56
NDAA307	0.71	0.87	1.3	0.87
NDAA152	-0.60	-0.69	-0.81	-0.69
NDAA200	0.72	0.55	0.39	0.55
NDAA273	-0.59	-0.81	-1.27	-0.81
NDAA151	0.74	0.76	1.24	0.76
NDAA298	-0.58	-0.63	-0.49	-0.63
NDAA330	-0.57	-0.55	-0.16	-0.55
NDAA326	0.75	0.62	0.31	0.62
NDAA116	0.75	0.48	-0.26	0.48
NDAA270	-0.56	-0.59	-0.47	-0.59
NDAA358	-0.54	-0.64	-0.79	-0.64
NDAA048	0.76	0.66	0.7	0.66
NDAA065	0.80	0.90	1.26	0.90
NDAA110	-0.51	-0.93	-2.05	-0.93
NDAA329	0.84	1.04	1.55	1.04
NDAA309	0.86	0.65	0.47	0.65
NDAA328	0.88	1.13	1.86	1.13
NDAA283	-0.47	-0.68	-1	-0.68
NDAA091	0.88	0.54	-0.14	0.54
NDAA085	0.89	0.59	0.11	0.59
NDAA304	0.91	0.78	0.66	0.78
NDAA286	-0.41	-0.41	-0.31	-0.41
NDAA184	1.01	0.66	0.01	0.66
NDAA294	1.02	0.84	0.5	0.84
NDAA187	1.03	1.09	1.33	1.09
NDAA458	-0.39	-0.37	-0.72	-0.37
NDAA331	-0.86	-0.70	-0.65	-0.70
NDAA057	-0.18	-0.50	-1.94	-0.50
NDAA077	0.63	0.50	0.82	0.50
NDAA188	-0.77	-0.68	-0.28	-0.68
NDAA354	0.67	0.71	0.91	0.71
NDAA372	-0.28	-0.33	-0.5	-0.33
NDAA165	-0.76	-0.67	-0.77	-0.67
NDAA142	0.61	0.39	0.14	0.39
NDAA255	-0.30	-0.53	-0.89	-0.53
NDAA052	0.68	0.73	1.03	0.73
NDAA119	-0.31	-0.43	-0.53	-0.43
NDAA113	-0.70	-0.46	-0.05	-0.46
NDAA044	0.59	0.66	0.69	0.66
NDAA214	-0.38	-0.35	-0.3	-0.35

NDAA104	-0.37	-0.56		-0.81	-0.56
NDAA415	-0.17	-0.41		-1.08	-0.41
NDAA215	0.58	0.43		0.04	0.43
NDAA094	0.61	0.55		0.49	0.55
NDAA321	-0.36	-0.55		-1.12	-0.55
NDAA341	-0.35	-0.41		-0.94	-0.41
NDAA251	-0.38	-0.37		-0.62	-0.37
NDAA318	0.55	0.49		0.23	0.49
NDAA368	-0.32	-0.57		-1.07	-0.57
NDAA426	0.33	0.43		0.57	0.43
NDAA443	-0.26	-0.53		-1.49	-0.53
NDAA409	0.36	0.46		0.72	0.46
NDAA120	0.53	0.53			0.53
NDAA385	-0.26	-0.26			-0.26
NDAB218	0.61		0.9		0.85
NDAB379	0.60		0.42		0.37
NDAB396	-0.71		0.99		0.94
NDAB322	0.77		1.05		1
NDAB455	0.91		0.36		0.31
NDAB197	0.80		0.61		0.56
NDAB174	0.61		0.73		0.68
NDAB147	0.61		0.66		0.61
NDAB258	0.61		0.72		0.67
NDAB454	0.63		0.69		0.64
NDAB308	-0.70		0.76		0.71
NDAB156	0.68		0.75		0.7
NDAB148	-0.73		0.4		0.35
NDAB366	0.59		0.74		0.69
NDAB123	0.81		0.94		0.89
NDAB529	0.35		0.58		0.53
NDAB195	0.61		0.79		0.74
NDAB225	0.72		1.36		1.31
NDAB286	0.59		1.54		1.49
NDAB414	0.81		0.61		0.56
NDAB304	0.70		-0.79		-0.84
NDAB487	0.63		-0.57		-0.62
NDAB494	0.62		-0.56		-0.61
NDAB153	-0.61		-0.28		-0.33
NDAB058	-0.48		-0.45		-0.5
NDAB502	-0.52		-1.33		-1.38
NDAB172	-0.59		-0.33		-0.38
NDAB460	-0.46		-0.86		-0.91
NDAB360	-0.74		-1.26		-1.31
NDAB117	-0.59		-1.1		-1.15
NDAB227	-0.52		-0.84		-0.89
NDAB465	-0.50		-1.09		-1.14
NDAB135	-0.82		-0.85		-0.9
NDAB203	0.60		-0.36		-0.41
NDAB208	-0.46		-0.47		-0.52
NDAB470	-0.55		-1.55		-1.6

NDAB184	-0.66	-0.95	-1
NDAB486	-0.54	-0.27	-0.32
NDAB367	0.78	-0.57	-0.62
NDAB281	-0.74	-1.2	-1.25

9.6 Significance (P-values) for fixed terms in all LMMs

Table 9-1: Significance (P-values) for P8 fatness during the breeding season in 2008

P8 fatness during breeding season 2008	**P8**
location	0.269
cohort	**<0.001**
lactating	**0.041**
calving date	0.133
line	0.150
height	0.543
FatvsLean	**0.044**
HiNFIvsLoNFI	**0.003**
nutrition	**<0.001**
line.nutrition	0.738
FatvsLean.nutrition	0.461
HiNFIvsLoNFI.nutrition	0.096
days-post-calving	**0.003**
days-post-calving.nutrition	0.094
days-post-calving.line	**0.026**
days-post-calving.FatvsLean	0.256
days-post-calving.HiNFIvsLoNFI	0.732

Table 9-2: Significance (P-values) for 3rd trimester P8 fatness analysis in 2007 and 2008; and analysis of the change in fatness during the breeding season in 2007 and 2008

	3rd trimester P8 2007	**3rd trimester P8 2008**	**P8 change 2007**	**P8 change 2008**
location	0.580	0.759	0.203	0.110
cohort		**<0.001**		**0.003**
line	**<0.001**	**<0.001**	0.250	0.660
height	**<0.001**	0.287	**0.032**	**0.006**
FatvsLean	**<0.001**	**0.010**	0.109	0.362
HiNFIvsLoNFI	0.065	**<0.001**	**0.072**	0.737
calving date	0.445	**<0.001**	0.769	**0.037**
pre-calving P8				
nutrition	0.206	**<0.001**	**0.009**	**0.006**
line.nutrition	0.809	0.886	0.770	**0.008**
FatvsLean.nutrition	0.261	0.833	0.331	0.116
HiNFIvsLoNFI.nutrition	**<0.001**	0.120	0.257	0.164

Table 9-3: Significance levels (P-values) analysis of liveweight change in 2008 and predicted yield (yield)

	P-values	
	liveweight change (kg)	yield (kg)
location	0.649	0.018
cohort	0.203	**<0.001**
calving date	**<0.001**	0.497
line	0.868	**<0.001**
FatvsLean	0.773	**0.028**
HiNFIvsLoNFI	0.800	**0.050**
nutrition	**<0.001**	**<0.001**
line.nutrition	0.801	0.126
FatvsLean.nutrition	0.435	0.153
HiNFIvsLoNFI.nutrition	0.468	0.145

Table 9-4: Significance level (P-values) for analysis of number of days to first ovulation post-calving (days-post-calving) in 2008

Progesterone analysis	**days-post-calving**
location	0.191
cohort	**<0.001**
line	0.137
FatvsLean	0.306
HiNFIvsLoNFI	0.885
calving date	**<0.001**
3rd trimester P8	0.600
nutrition	0.113
line.nutrition	0.213
FatvsLean.nutrition	0.354
HiNFIvsLoNFI.nutrition	0.721

Table 9-5: Significance (P-values) of terms in analysis of calf production (birth weight, growth rate, weaning weight, DTC) in 2007

Calf production 2007	**birth weight**	**growth rate**	**weaning weight**	**DTC**
location	0.478	0.997	0.874	0.660
calving date	**<0.001**	0.213	**<0.001**	
calf sex	**0.067**	**0.062**	**0.047**	
line	**0.010**	**<0.001**	**<0.001**	0.687
height	0.770	**0.055**	**0.038**	
FatvsLean	0.253	0.324	0.213	0.576
HiNFIvsLoNFI	0.314	0.518	0.461	0.462
dam birth date	0.190	0.220	0.431	**0.013**
3rd trimester P8	0.819	0.649	0.641	
nutrition	0.986	**0.021**	**0.024**	0.139
line.nutrition	0.458	0.973	0.871	0.463
FatvsLean.nutrition	0.978	0.954	0.978	0.992
HiNFIvsLoNFI.nutrition	0.670	0.924	0.841	0.794

Table 9-6: Significance (P-values) of terms in analysis of calf production (birth weight, growth rate, weaning weight, DTC, calf P8 at weaning) in 2008

Calf Production 2008	**birth weight**	**growth rate**	**weaning weight**	**DTC**	**calf P8 at weaning**
location	0.553	0.998	0.712	0.501	0.594
cohort	0.116	**<0.001**	**0.005**	0.325	0.893
calving date	**<0.001**	0.676	**<0.001**		**0.001**
multiple birth	**<0.001**	0.393	0.298	**<0.001**	0.301
calf sex	**<0.001**	**<0.001**	**<0.001**		**0.006**
line	**<0.001**	**<0.001**	**<0.001**	0.495	0.302
height	0.171	0.352	0.287		0.113
FatvsLean	**0.065**	0.631	0.545	0.157	0.119
HiNFIvsLoNFI	0.781	0.269	0.262	0.845	0.627
dam birth date	0.707	0.917	0.741	0.452	0.352
3rd trimester P8	0.542	0.730	0.639		0.393
nutrition	0.611	**0.017**	**0.039**	**0.057**	**0.007**
line.nutrition	0.227	0.334	0.466	0.753	0.237
FatvsLean.nutrition	0.854	0.995	0.831	0.429	0.524
HiNFIvsLoNFI.nutrition	**0.063**	0.723	0.823	0.539	0.505

Table 9-7: Level of significance (P-values) for efficiency parameters; DM disappearance (kg DM/head/day), ME MJ disappearance (MJ ME/head/day) and MJ ME disappearance per kg weaned

Efficiency parameters	**DM disappearance**	**MJ ME disappearance**	**MJ ME per kg weaned**
%lact	0.064	0.073	0.279
nutrition	**0.007**	**0.010**	**0.050**
line	0.963	0.867	0.667
HiNFIvsLoNFI	0.196	0.204	0.098
FatvsLean	0.691	0.140	0.706
nutrition.line	0.522	0.480	0.706
nutrition.HiNFIvsLoNFI	0.984	0.713	0.755
nutrition.FatvsLean	0.283	0.397	0.217

Table 9-8: Significance level (P-values) for analysis of pre-calving blood parameters in 2007

Pre-calving 2007	**BHB**	**glucose**	**acetate**	**NEFA**	**leptin**	**IGF1**	**insulin**	**GH**
location	0.669	0.569	0.700	0.259	0.723	0.475	**0.049**	0.103
line	0.540	0.959	**0.016**	**<0.001**	**<0.001**	0.536	0.239	**<0.001**
height	**0.002**	0.785	0.510	0.104	**0.025**	**<0.001**	**0.031**	**0.005**
FatvsLean	**0.007**	0.260	0.291	**0.026**	**0.003**	**0.032**	**0.002**	**<0.001**
HiNFIvsLoNFI	**<0.001**	0.187	0.527	**0.028**	0.762	0.288	**0.004**	**<0.001**
calving date	0.851	0.779	**0.058**	**<0.001**	0.606	0.573	0.157	0.256
3rd trimester P8	**0.008**	0.591	0.810	**<0.001**	**<0.001**	**<0.001**	0.436	**<0.001**
nutrition	**0.043**	0.581	**0.007**	0.279	0.267	**0.040**	0.627	0.349
line.nutrition	0.924	0.915	0.536	0.612	0.667	0.979	**0.035**	0.119
FatvsLean.nutrition	**0.056**	0.778	0.857	0.788	0.456	0.440	0.987	**0.001**
HiNFIvsLoNFI.nutrition	**0.027**	0.073	0.217	**0.040**	0.187	0.111	**0.008**	0.985

Table 9-9: Significance level (P-values) for analysis of pre-calving blood parameters in 2008

Pre-calving 2008	**BHB**	**glucose**	**acetate**	**NEFA**	**leptin**	**IGF1**	**insulin**	**GH**
location	0.628	**0.053**	0.567	0.996	0.235	0.178	0.244	0.549
cohort	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	0.508	**0.033**	**<0.001**
line	0.586	**<0.001**	0.462	**0.026**	0.088	**0.001**	0.099	0.419
height	0.261	0.785	0.183	**0.002**	**0.005**	**0.026**	**0.041**	0.207
FatvsLean	**0.006**	0.957	0.946	**0.021**	**0.043**	0.105	0.220	0.942
HiNFIvsLoNFI	0.265	**0.065**	0.449	0.702	0.936	0.611	0.139	0.585
calving date	**<0.001**	0.198	0.150	**<0.001**	**0.001**	0.097	**0.003**	**<0.001**
3rd trimester P8	**<0.001**	0.096	**0.046**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
nutrition	**0.011**	**<0.001**	0.149	0.519	**0.026**	0.246	**0.023**	0.087
line.nutrition	0.369	0.741	0.520	0.659	0.748	0.972	0.361	0.872
FatvsLean.nutrition	**0.054**	0.501	0.975	0.138	0.159	0.472	0.657	0.228
HiNFIvsLoNFI.nutrition	0.984	0.300	0.779	0.322	0.939	0.771	0.527	0.329

Table 9-10: Significance level (P-values) for analysis of post-calving blood parameters in 2007

Post-calving 2007	**BHB**	**glucose**	**acetate**	**NEFA**	**leptin**	**IGF1**	**insulin**	**GH**
location	0.102	0.740	**0.038**	0.068	0.235	**0.038**	**0.003**	0.330
lactating	**0.019**	**0.060**	**0.004**	**0.017**	0.786	0.416	0.757	0.983
calving date	0.518	0.570	0.818	**0.001**	0.700	**<0.001**	0.394	0.219
line	0.783	**0.008**	**<0.001**	0.177	**0.022**	0.162	0.452	**0.016**
height	0.546	0.887	0.423	0.115	0.349	0.338	0.513	0.576
3rd Trimester P8	0.717	0.875	0.929	0.954	**<0.001**	0.834	0.843	**0.066**
FatvsLean	**0.058**	0.618	0.362	0.977	**0.063**	0.113	0.636	0.206
HiNFIvsLoNFI	0.510	**0.072**	0.682	0.443	0.203	0.789	**0.072**	0.577
nutrition	0.634	0.845	0.298	0.150	0.192	0.724	0.095	0.453
line.nutrition	0.745	0.542	0.284	0.350	0.526	0.091	0.761	0.302
FatvsLean.nutrition	0.695	0.372	0.106	0.190	**0.014**	0.474	0.446	0.470
HiNFIvsLoNFI.nutrition	0.263	0.619	**0.050**	0.322	0.099	0.722	**0.035**	0.145
pre-calving measure	0.225	0.297	**0.002**	0.189	**<0.001**	**0.002**	**0.045**	**<0.001**
days-post-calving	0.334	**0.001**	0.209	**<0.001**	0.171	0.093	0.761	0.892
days-post-calving.nutrition	0.521	0.605	0.184	**0.011**	**0.045**	0.714	0.415	0.131
days-post-calving.line	0.882	0.815	0.776	0.374	0.395	0.957	0.272	0.066
days-post-calving.FatvsLean	0.844	0.105	0.913	**0.002**	0.260	0.793	0.797	0.950
days-post-calving.HiNFIvsLoNFI	0.784	0.462	0.830	0.614	0.281	0.940	0.487	0.384
days-post-calving.line.nutrition	0.901	0.142	0.975	0.093	0.133	0.068	**0.040**	0.511
days-post-calving. FatvsLean.nutrition	0.228	0.103	**0.003**	0.196	0.786	0.817	**0.019**	0.637
days-post-calving.HiNFIvsLoNFI.nutrition	0.529	0.388	0.458	0.093	0.373	0.917	**0.010**	0.125

Table 9-11: Significance level (P-values) for analysis of post-calving blood parameters in 2008

Post-calving 2008	**BHB**	**glucose**	**acetate**	**leptin**	**IGF1**	**insulin**	**GH**
location	0.216	0.107	0.599	0.612	0.843	0.441	**0.006**
cohort	**0.014**	**0.048**	0.187	**<0.001**	**<0.001**	0.260	**<0.001**
lactating	**<0.001**	**0.024**	**<0.001**	**<0.001**	**<0.001**	0.582	0.400
calving date	0.431	**0.015**	0.543	**0.001**	**0.008**	0.548	**0.015**
line	0.724	**0.002**	0.104	0.318	0.138	0.123	**0.001**
height	**0.050**	0.479	0.384	0.142	0.127	0.806	0.549
3rd trimester P8	0.115	0.102	0.282	**<0.001**	0.205	**0.002**	**<0.001**
FatvsLean	0.979	0.788	0.232	0.592	0.439	0.371	0.103
HiNFIvsLoNFI	0.473	0.663	**0.020**	0.933	0.950	0.763	0.108
nutrition	0.186	**0.036**	0.448	**<0.001**	0.671	0.259	**<0.001**
line.nutrition	0.433	0.901	0.946	0.074	0.520	**0.055**	**0.048**
FatvsLean.nutrition	0.265	0.837	0.308	0.170	0.864	0.128	0.709
HiNFIvsLoNFI.nutrition	0.262	0.420	0.622	0.301	0.440	0.477	0.994
pre-calving measure	**0.022**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.006**	**<0.001**
days-post-calving	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.203	**<0.001**
days-post-calving.nutrition	**<0.001**	0.926	**0.043**	0.476	0.070	**<0.001**	0.574
days-post-calving.line	0.796	0.720	0.769	0.861	0.575	0.942	**0.034**
days-post-calving. FatvsLean	**0.002**	0.411	**<0.001**	0.936	0.561	0.650	**0.054**
days-post-calving.HiNFIvsLoNFI	0.571	0.612	0.568	0.080	0.125	0.579	0.276
days-post-calving.line.nutrition	0.543	0.972	0.699	0.451	0.614	0.880	0.472
days-post-calving. FatvsLean.nutrition	0.813	0.891	0.272	0.772	0.393	0.101	0.649
days-post-calving.HiNFIvsLoNFI.nutrition	0.777	0.616	**<0.001**	0.155	0.457	0.642	**0.046**

Table 9-12: Significance levels (P-values) for analysis of rate of change of blood parameter compared to changing fatness in 2008

Rate of change blood parameter vs. change in P8	**BHB**	**glucose**	**acetate**	**GH**	**IGF1**	**leptin**	**insulin**
location	0.842	**0.001**	0.129	0.314	0.539	0.413	0.249
cohort	0.878	0.402	0.138	**0.004**	0.408	0.826	0.059
Line	0.348	0.074	0.173	0.518	0.937	0.067	0.656
height	0.117	0.743	0.401	0.376	**0.036**	0.130	0.282
FatvsLean	0.317	0.250	0.768	0.132	0.671	0.854	0.389
HiNFIvsLoNFI	0.406	0.079	0.533	0.967	0.150	0.200	0.644
calving date	0.597	**0.023**	**<0.001**	**0.008**	0.198	0.308	**0.013**
nutrition	**0.004**	0.200	0.111	0.750	0.423	0.786	**0.034**
line.nutrition	0.875	0.639	0.117	0.349	0.516	0.270	0.944
FatvsLean.nutrition	0.620	0.791	**0.022**	0.547	0.135	0.780	0.382
HiNFIvsLoNFI.nutrition	0.862	0.946	0.060	0.203	0.427	0.618	0.825
P8 change	0.412	0.209	0.609	0.205	0.715	**0.033**	0.839

	UASMS1						
	leptin		**EMA**		**IMF**		**P8**
	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	post-calving (day14)
location	0.048	0.557	0.028	**<0.001**	0.013	0.460	0.291
cohort	**<0.001**	**<0.001**	0.012	**<0.001**	0.039	**<0.001**	**<0.001**
height	0.093	0.072	**0.011**	**0.016**	0.092	0.266	0.499
calving date	0.738	0.615	0.626	0.414	0.038	0.459	0.864
line	0.971	0.101	0.239	0.116	0.112	0.903	0.632
nutrition	0.031	<0.001	0.017	**<0.001**	**0.027**	**<0.001**	**<0.001**
UASMS1	**<0.001**	**<0.001**	0.452	0.867	0.073	0.652	0.929
line.nutrition	0.326	0.119	0.579	0.938	0.402	0.651	0.704
line.UASMS1	0.386	0.727	0.667	0.539	0.062	0.895	0.93
nutrition.UASMS1	0.142	0.856	0.249	0.255	0.015	0.868	0.110
line.nutrition.UASMS1	0.168	0.562	0.079	0.663	0.090	0.947	0.618

	UASMS2						
	leptin		**EMA**		**IMF**		**P8**
	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	post-calving (day14)
location	0.254	0.348	**0.027**	**<0.001**	**0.026**	0.344	0.332
cohort	**<0.001**	**<0.001**	**0.004**	**<0.001**	**0.045**	**<0.001**	**<0.001**
height	0.391	0.704	**0.010**	**0.006**	0.847	0.638	0.398
calving date	0.804	0.705	0.343	0.164	**0.031**	0.448	0.983
line	0.982	0.21	0.466	0.368	0.095	0.972	0.558
nutrition	**0.050**	**<0.001**	**0.022**	**<0.001**	**0.055**	**<0.001**	**<0.001**
UASMS2	0.873	0.526	0.509	0.395	0.808	0.875	0.288
line.nutrition	0.277	0.112	0.384	0.974	0.295	0.579	0.952
line.UASMS2	0.942	0.613	0.064	0.29	0.035	0.085	0.166
nutrition.UASMS2	0.204	0.867	0.97	0.165	0.279	0.868	0.319
line.nutrition.UASMS2	0.943	0.87	0.927	0.236	0.392	0.38	0.706

	E2FB

	Leptin		EMA		IMF		P8
	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	post-calving (day14)
location	0.139	0.412	**0.031**	**<0.001**	**0.019**	0.351	0.279
drop	**<0.001**	**<0.001**	**0.005**	**<0.001**	**0.055**	**<0.001**	**<0.001**
height	0.094	0.315	**0.003**	**0.002**	0.461	0.865	0.219
calving date	0.497	0.736	0.472	0.251	0.087	0.348	0.721
line	0.748	0.125	0.383	0.257	0.069	0.756	0.439
nutrition	**0.015**	**<0.001**	**0.021**	**<0.001**	0.063	**<0.001**	**<0.001**
E2FB	**<0.001**	**<0.001**	0.771	0.508	0.593	0.662	0.476
line.nutrition	0.871	0.211	0.494	0.730	0.331	0.681	0.857
line.E2FB	0.193	0.811	0.402	0.556	0.018	0.247	0.765
nutrition.E2FB	0.171	0.168	0.763	0.679	0.46	0.838	0.604
line.nutrition.E2FB	0.141	0.840	0.639	0.919	0.170	0.387	0.395

	E2JW						
	Leptin		EMA		IMF		P8
	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	post-calving (day14)
location	0.839	0.427	0.155	**0.010**	0.343	0.424	0.254
drop	0.187	0.091	**0.026**	**0.013**	0.188	**0.011**	**<0.001**
height	0.060	0.247	0.513	0.073	0.064	0.608	0.536
calving date	0.382	0.277	0.281	0.935	0.967	0.835	0.196
nutrition	0.113	0.066	0.118	0.013	0.910	0.033	0.011
E2JW	0.758	0.840	0.237	0.687	0.639	0.87	0.151
nutrition.E2JW	0.555	0.108	0.409	0.166	0.800	0.054	0.971

Table 9-13 (above): Significance level (P-value) for associations between SNPs (UASMS1, UASMS2, E2FB, and E2JW) with pre- and post-calving (predicted for day 14 post-calving) leptin, EMA, IMF and post-calving P8 (P8)

SNP	Leptin		EMA		IMF		P8
	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	pre-calving	post-calving (day14)	post-calving (Day14)
UASMS1	**<0.001**	**<0.001**	0.452	0.867	0.073	0.652	0.929
UASMS2	0.873	0.526	0.509	0.395	0.808	0.875	0.288
E2FB	**<0.001**	**<0.001**	0.771	0.508	0.593	0.662	0.476
E2JW	0.758	0.84	0.237	0.687	0.639	0.87	0.151

CHAPTER 10. BIBLIOGRAPHY

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